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TITOLO TESI

Disentangling the effects of age and global change on *Pseudotsuga menziesii (Mirb.) Franco* growth and water use efficiency

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Abstract

The recent alterations in forests growth could be the result of a combination of different climatic and non-climatic factors, as rising atmospheric [CO_2], temperature fluctuations, atmospheric nitrogen deposition and drought stress. This study tests the potential effects of global change on trees, assessing the relative importance and functional relationships between environmental drivers and long-term growth trend, as well as physiological response. To investigate such effects, we applied Generalized additive models (GAMs) technique, decoupling the non-linear age related effect from co-occurring environmental effects on basal area increments (BAI) series and isotope proxies ($\delta^{13}C$ and $\delta^{18}O$). Two Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) chronosequence were considered; the first one, comprises four different age classes (age 65-, 80-, 95- and 120-) and is a even-aged stands plantation located in Italy, while the second one is an old-growth Californian stand, with three age classes (age 100, 200, 300). Results show a 22.9% decrease of the general BAI growth trend over last decades for the Italian Douglas-fir chronosequence, when the age-size non-linear effect was removed. A related trend in water use efficiency ($iWUE=A_{max}/g_{s,}$ the ratio between photosynthetic assimilation and stomatal conductance) was observed in the same period. Thus, through the application of the so called dual isotope approach, was possible to attribute to a reduction in A_{max} the cause of such a trend, probably driven by a reduction in N deposition. On other hand, BAI trend accounted for the Californian old-growth stand shows an increase of roughly the 60% since the 1960, which was found to be mostly determinate by a strong effect of atmospheric [CO_2]. These founding highlight how this species has been affected by global change impact in both sits and provide important insights on its future behavior, potentially driving management choices.

Key words: *Pseudotsuga menziesii,* BAI, iWUE, long-term trends, global change, GAMs, isotope dual approach.

Summary

Abstract

General introduction

The ongoing global changes are intimately linked to the increase in the concentration of greenhouse gases in the atmosphere, due to anthropogenic emission. The lower degree of infrared solar radiation that can be dissipated out of the earth system results in an increase in global average temperatures, attended to reach levels included between 2°C up to 4.5°C degrees by the end of this century, as well as an alteration of extent and distribution of precipitation (IPCC 2014). The main greenhouse gas, CO_2, has reached today a concentration of 406 μmol mol^{-1}, the highest in the last 650000 years. Over the past 250 years, atmospheric CO_2 has been increased globally by the 30% from the concentration of 285 ppm in the pre-industrial era, with an exponential progression. The human carbon source, resulting by fossil fuel combustion and land use change, is estimated in 8.8 Gt C y-1. The main sinks, which absorb actively carbon from atmosphere, are oceans and terrestrial ecosystems, able to remove roughly 2 Gt C each one every year. The extent of the terrestrial sink, manly represented by forest ecosystems, is directly and indirectly influenced by global change. The temperature variations can increase (enlarging the vegetative period)(Menzel and Fabian, 1999) or decrease (water stress due to excessive evapo-transpiration)(Allen et al. 2010) plants' photosynthetic assimilation. On the other hand, the greater amount of CO_2 available to plants photosynthesis could acts positively on the growth capacity of trees, amplifying their mitigating effect (Ainsworth and Long 2005). For what concern the northern hemisphere and temperate forests in particular was observed, both through satellite direct measurement of NDVI (Normalized Difference Vegetation Index) and ground-based data, an increase in net primary productivity (NPP, gross primary productivity minus autotrophic respiration, GPP-R_a) of 20% in the last decades of the 20th century (Boisvenue and Running 2006). The causes were attributed for a 50% to direct effects of forest management, for 33% to the direct and indirect effects caused by global change and for 8-17% to historical effects related to age stands dynamics (Vetter et al. 2005). Contrarily, more recent observations seem to highlight an inversion of this trend. The NPP in the first decade of the

new millennium suffers a global decrease, probably due to the drought events induced by higher temperatures (Zhao & Running, 2010). A better understanding of how [CO_2] acts on forests both directly and through the improvement of positive or negative feedbacks, as which one involved temperature and water stress process or trees growth enhancement, is of crucial importance. Indeed, it determine the capacity of realize predictive reliable models on the future global change entity.

Another key factor determining these anomaly in forest growth has been identified in the potential fertilizing effect of atmospheric nitrogen depositions (Hyvönen et al., 2007), caused by atmospheric pollution of nitrogen oxidized (NO_y deriving from combustion) and of reduced form (NHx deriving from agricultural fertilization). Discounted the age-related effect, a quasi-linear relationship between N depositions and net ecosystem productivity (NEP, difference between gross primary productivity and total ecosystem respiration, GPP-R) has been hypothesized (Magnani et al. 2007), caused by the direct nitrogen canopy uptake which can bypass bacterial competition in the soil and the relative increase in heterotrophic respiration (R_h ,source of C). It would increase C sequestered by plants in temperate forest ecosystems (typically N-limited) increasing their sink effect. Furthermore, when long-term analysis on trees growth is performed, it should be taken into consideration also that forests normally display a progressive reduction in productivity as stand age increase. For example, Aboveground Productivity (P_a, one of the components of NPP) is influenced by age-size related dynamics in the leaf area index (LAI, defined as the relationship between the photosynthetically active leaf surface and the surface of the soil on which the leaves are projected). After a juvenile phase of expansion, determining an increase in time of P_a, LAI reaches a culmination at stand canopy closure, exceeded which the increase in inter-tree competition for light, water and nutrients progressively reduces P_a (Ryan and Yoder, 1997). Another component of this age-effect is explained by the hydraulic limitation hypothesis (HLH) which relates productivity decrease and tree dimension. The gradual increase in hydraulic resistance with the increase of the height of the stem, the length of the branches and the thickness of the roots, would cause an enhancement in the internal water potential difference between roots and shoots, if hydraulic leaf

conductance (k) is maintained constant. Instead, a k reduction is observed with trees ageing, caused by a decrease of stomatal conductance (g_s). This link between hydraulic resistances and the degree of stomatal closure affect net photosynthesis rates, decreasing the amount of CO_2 which could be assimilated in relation to LAI (*Gower et a. 1996*) .The maintenance of a almost constant leaf potential, obtained through the reduction of stomatal conductance is a compromise between the photosynthesis, water transport at greater heights (which would be more effective at more negative leaf water potential) and cavitations avoidance (embolisms caused by the vascular system water chain brake) which would be suffered by the xylem if extremely negative water potentials would be reached (Tyree and Sperry, 1988; Magnani et al. 2000).

Separate these age-size related effects from the co-occurring environmental changes effects affecting trees growth, is virtually impossible if a single age-class is considered because both of them are time correlated (Bowmanet al., 2013). Indeed, exogenous effects, as climate-related covariates or biogeochemical pollutants impact, varying along calendar year while age-size effects (endogenous), varying along cambial age. To overcome such inter-correlation issues is possible to apply a sampling strategy aimed on the collection of a wide range of age-classes from a multi-age stand or from different even-aged stands growing in comparable environmental condition, but established in different dates. This chronosequence-based approach sensu Walker et al. (2010) allow to assess the effects of changing environmental conditions affecting tree growth (i.e, rising [CO_2], temperature or water availability) trough the deviation from the attended age-related mean trend . Based on this eco-physiological and environmental background , the first objective of this PhD thesis is to evaluate the possible impact of global change affecting two Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) chronosequences, separating age -related growth changes from environmentally-driven long-term growth trends superimposed on them. The second aim is to test the possibility to disentangling among singular environmental factors which one have mostly determined long-term growth and eco-physiological response trend variations, with the idea of

highlight potential vulnerability or strength points exhibited by this species, also in a future adaptation prospective of this species to the Italian environment.

Bibliography

Ainsworth, Elizabeth A., and Stephen P. Long. 2005. "What Have We Learned from 15 Years of Free-Air CO2 Enrichment (FACE)? A Meta-Analytic Review of the Responses of Photosynthesis, Canopy Properties and Plant Production to Rising CO2." *New Phytologist* 165 (2): 351–71. doi:10.1111/j.1469-8137.2004.01224.x.

Allen, Craig D., Alison K. Macalady, Haroun Chenchouni, Dominique Bachelet, Nate Mcdowell, Michel Vennetier, Thomas Kitzberger, et al. 2010. "A Global Overview of Drought and Heat-Induced Tree Mortality Reveals Emerging Climate Change Risks for Forests." *Forest Ecology and Management* 259 (4): 660–84. doi:10.1016/j.foreco.2009.09.001.

Bowman, David M.J.S., Roel J.W. Brienen, Emanuel Gloor, Oliver L. Phillips, and Lynda D. Prior. 2013. "Detecting Trends in Tree Growth: Not so Simple." *Trends in Plant Science* 18 (1). Elsevier Ltd: 11–17. doi:10.1016/j.tplants.2012.08.005.

Gower ST, McMurtrie RE, Murty D (1996) Aboveground net primary production decline with stand age: potential causes. Trends Ecol Evol Res 11: 378-382.

Hyvönen, Riitta, Göran I Agren, Sune Linder, Tryggve Persson, M Francesca Cotrufo, Alf Ekblad, Michael Freeman, et al. 2007. "The Likely Impact of Elevated [CO2], Nitrogen Deposition, Increased Temperature and Management on Carbon Sequestration in Temperate and Boreal Forest Ecosystems: A Literature Review." *The New Phytologist* 173 (3): 463–80. doi:10.1111/j.1469-8137.2007.01967.x.

IPCC. 2014. *Summary for Policymakers*. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. doi:10.1017/CBO9781107415324.

Magnani, F., M. Mencuccini, and J. Grace. 2000. "Age-Related Decline in Stand Productivity: The Role of Structural Acclimation under Hydraulic Constraints." *Plant, Cell and Environment* 23 (3): 251–63. doi:10.1046/j.1365-3040.2000.00537.x.

Magnani, Federico, Maurizio Mencuccini, Marco Borghetti, Paul Berbigier, Frank Berninger, Sylvain Delzon, Achim Grelle, et al. 2007. "The Human Footprint in the Carbon Cycle of Temperate and Boreal Forests." *Nature* 447 (7146): 848–50. doi:10.1038/nature05847.

Menzel, Annette, and Peter Fabian. 1999. "Growing Season Extended in Europe." *Nature* 397 (6721): 659–659. doi:10.1038/17709.

Ryan, Michael G., and Barbara J. Yoder. 1997. "Hydraulic Limits to Tree Height and Tree Growth." *BioScience* 47 (4): 235–42. doi:10.2307/1313077.

Tyree, M T, and J S Sperry. 1988. "Do Woody-Plants Operate Near the Point of Catastrophic Xylem Dysfunction Caused By Dynamic Water-Stress - Answers From A Model." *Plant Physiology* 88 (3): 574–80. doi:10.1104/pp.88.3.574.

Vetter, Mona, Christian Wirth, Hannes Böttcher, Galina Churkina, Ernst Detlef Schulze, Thomas Wutzler, and Georg Weber. 2005. "Partitioning Direct and Indirect Human-Induced Effects on Carbon

Sequestration of Managed Coniferous Forests Using Model Simulations and Forest Inventories." *Global Change Biology* 11 (5): 810–27. doi:10.1111/j.1365-2486.2005.00932.x.

Walker, Lawrence R., David a. Wardle, Richard D. Bardgett, and Bruce D. Clarkson. 2010. "The Use of Chronosequences in Studies of Ecological Succession and Soil Development." *Journal of Ecology* 98 (4): 725–36. doi:10.1111/j.1365-2745.2010.01664.x.

Chapter I - Disentangling the effects of age and global change on Douglas-fir growth

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1. Introduction

Over recent decades, significant changes in forest growth have been observed, particularly in Europe, which have been interpreted as a result of the ongoing global change (Boisvenue and Running 2006; Zhao and Running 2010) . However, the main drivers and functional basis of this have not been ascertained. The potential effect of atmospheric CO_2 fertilisation during the Antrophocene is one of the most widely discussed explanations, based on the expected stimulation in photosynthetic rates at plant and ecosystem scale, with a positive effect on net primary productivity (NPP). Only a few experiments have gathered evidence to test this hypothesis (Ainsworth and Long 2005), the majority of which did not find a clear relationship between CO_2 and growth enhancement (Lévesque et al. 2014). Other studies have reported such an increase, although stressing the importance of concomitant related factors, for example disturbance history or an increasing vegetative period (McMahon et al. 2010). Indeed, interactions with other environmental variables such as atmospheric nitrogen deposition (Magnani et al. 2007) are expected to play a determinant role especially in resource-limited environments. Moreover, a parallel increase in transpiration rates as a result of increasing temperatures could negate this positive effect, in particular in drought-prone areas (Gómez-Guerrero et al. 2013)There is therefore a pressing need to understand which key drivers have been affecting forest growth rates, and quantify the magnitude

of their effects. Even in the absence of controlled experiments, the analysis of long-term trends in tree growth can help elucidate the relationship with environmental factors, as variations in the growth pattern of a tree are the result of changing conditions, as well as ontogenetic processes (Babst et al. 2014). Tree-ring widths are a direct measure of stem growth, hence the inspection of this time series provides a reliable and datable source of data that can be used to investigate high and low-frequency variability in forest growth trends. In order to highlight the environmental-related signals enclosed in the tree-ring series, however, the superimposed age-related signal must be first removed. An age-related decline in ring widths is generally observed with increasing age, as a result of biological processes as well as geometrical constraints; basal area increments, on the contrary, generally display an increase with age, followed by a gradual stabilization. Canonical procedures applied in dendrochronological studies remove this age-related biological trend through the application of de-trending techniques, such as spline or negative exponential fitting (Peters et al. 2015). However, a consequence of this is the depletion of low-frequency signals associated to tree-ring series (Cook et al. 1995). Preserving low-frequency variations is of fundamental importance if the objective of the analysis is to investigate long-term trends (Esper et al. 2002)**.** In this study, Generalised Additive Models (GAMs) were used as a tool to detect and separate the effect of different variables, both biological (i.e. age) and environmental, and to determine tree-rings series trends on a Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) chronosequence. This non-linear regression technique is a *ceteris paribus* form of analysis, looking at the effect of a single factor while keeping remaining factors constant (Rita et al. 2016). Hence, it is possible to take into account tree age effects as a simple additive variable and look at the parallel effects of other environmental covariates (Federal et al. 2015). Therefore, such a model can provide an alternative to the traditional de-trending procedures, with the advantage to retain low frequency variability in the series. In addition, it deals with the non-linearity of the relationship between the response and the explanatory variables.

The aim of the present study is:

(i) to evaluate the possibility of separating age/size effects from environmentally-induced long-term growth trends, avoiding the use of common de-trending methods and

(ii) to understand if Douglas-fir is affected by the changing environmental pressure in a long-term perspective, looking at which variable or combination of variables drives the observed change in growth rates.

2 Materials and methods

2.1 Study area

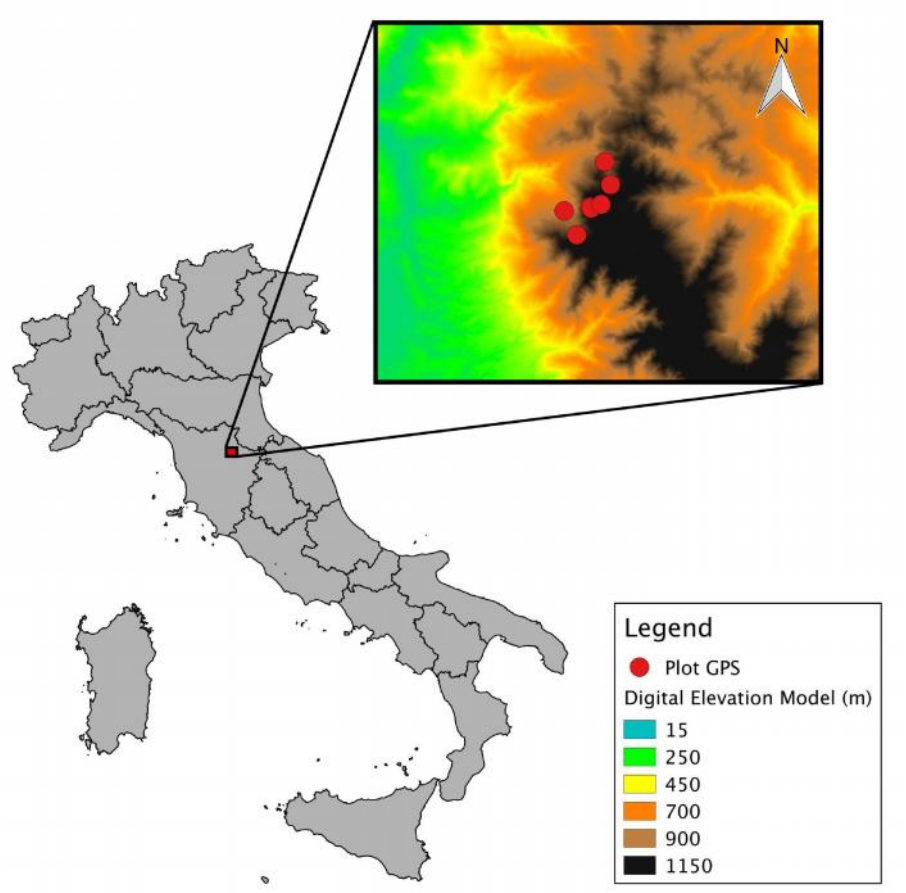


Fig.1 Map shows the location of the seven plots sampled (red dots). Different colors are related to different elevations (m, a.s.l).

This study was performed in a Douglas-fir plantation located in the Vallombrosa Forest, in the Apennine mountain range near Florence, Italy (43°43'59.6"N 11°33'16.9"E). The region has a Mediterranean climate without significant summer droughts, and the mean annual precipitation is

approximately 1400 mm, of which less than 10% occurs in the summer months (72.48 mm). The mean annual temperature is 9.8°C. The soils, derived from the Macigno del Chianti sandstone series, vary between Humic Dystrudept and Typic Humudept (USDA Soil Survy Staff, 1999) in the younger and older stands, respctively, indicating similar soil conditions at the sites. Douglas-fir is a non-indignous evergreen species and was imported from the Pacific Coast of the United States during the last decades of the 19th century. It was chosen for the present study because of its high economic importance. The sampled areas are part of the experimental permanent plot network managed by CREA Research Centre for Forestry and Wood, and include the oldest experimental plots established in Italy at the beginning of the 20th century (Pavari 1916).

A chronosequence of plots was selected for the study; a chronosequence is here defined as a set of even-aged stands growing under the same environmental conditions and differing only for their age (Walker et al. 2010). The chronosequence comprises four different age classes (65, 85, 100 and 120 years), covering the longest temporal extension that is possible to achieve in Italy for this species. The summary characteristics of the four age classes are summarized in Table 1. Seven plots were sampled that were consistent for management, aspect and elevation. Two plots were selected for each age class, in order to ensure replication; however this was not possible for the oldest class, as only one of this age was present in the area. In all sites, only dominant trees were chosen for the analysis. Data from repeated forest inventories at the sites ensured the permanence of their dominant status, thus partially avoiding potential sampling biases which occur when the currently largest-diameter trees are wrongly considered to have always been in the dominant class (Cherubini et al. 1998). However, the growth of shade intolerant trees is very much dependent by stand density, especially in even-aged stands. Even if the trees sampled have been maintaining the dominant status and thus should be considered exempt by growth suppression deriving to competition effect, is not possible to exclude the presence of positive influence of thinning (i.e release effect) (Fernàndez-de-Una et al. 2016).

Tab. 1| Mean characteristics of the Douglas fir chronosequence plots.

	Age-class						
	120	100			85	65	
Plot (code)	1	2	3	4	5	6	7
Max age (yrs)	126	101	102	86	86	69	69
Elevation (a.s.l.)	900	1100	1009	1095	1280	1113	1113
Esposition	N	N	SW	NE	NE	SW	SW
Dominant diameter (cm)	--	60.8	67.5	58.2	61,5	50.3	43.3
Dominant high (m)	--	47.2	54.4	49.9	44.3	39.9	39.8
Stand density (n°ha-1)	30*	375	380	360	280	600	550
Trees sampled (n°)	5	5	5	5	5	5	5

*for the oldest plot only 30 plants are left standing

2.2 Tree-ring data

In the spring and fall of 2013, 35 trees were sampled, five from each of the aforementioned plots. One single core was extracted at breast height from each tree with a 5.1 mm Pressler borer (Haglöf, Sweden). The extracted cores were then air-dried and polished with progressively finer sandpaper (60- to 300-grit), so as to distinguish annual ring boundaries. Ring width series were measured on pictures taken with a long-focal high definition camera (Canon, Japan) with the COORECORDER image software analyser (Cybis Elektronik and Data AB) with 0.01 mm precision. Samples were visually cross-dated against a reference curve, between and within the series using a correlation coefficient, Gleichläufigkeit values and Student's t-test as indices. The closest tree ring chronology available in the International Tree Rings Data Base (ITRDB) was used as a reference for pointer year detection; the selected dataset (Schweingruber, F.H. - Mount Falterona - ABAL - ITAL008) refersd to an *Abies alba* chronology from Mount Falterona (23 km from Vallombrosa). As a further check, a reference curve was developed using the Douglas-fir dataset itself by the 'leave-one-out' methodology, starting from samples with a high correlation with the previous reference curve used. Therefore, the quality of cross-dating was checked and cross-correlation analysis was performed using the CDENDRO software (Cybis Elektronik and Data AB) and the R dplR pakage (Bunn 2008). Where the extracted core did not reach the pith of the tree, the length to the centre was

estimated using the curvature of the last complete ring, and the number of missing rings was calculated by dividing this distance by the last five-year ring width average (Applequist et al. 1958). These values were then checked against the year of plantation establishment, according to the forest management plan.

Subsequently, the raw-ring widths recorded were converted into basal area increment (BAI), as the latter allows to compensate for the age effect associated with the geometry of stems, especially at young age, while preserving low-frequency variability (Biondi 1999). Moreover, BAI is considered a better proxy of growth compared with radial increments. It was calculated as:

$$BAI = \pi (r^2_t - r^2_{t-1}) \quad (1)$$

where r_t is the stem radius in a given year and r_{t-1} is the value corresponding to the previous year.

2.3 Environmental data

Daily records of mean, maximum and minimum temperatures and precipitation were obtained from the Regional Hydrological Service of the Tuscany Region (SIR). Measurements for the 1922-2013 period were derived from the closest weather station, located at less than 3 km from sampled plots, and integrated with the dataset obtained by Gandolfo-Sulli (1990) for the 1897-1922 period.

Mean annual data of air CO_2 concentration were obtained from the NOAA Earth System Research Laboratory, as recorded at the Mauna Loa observatory in Hawaii from 1959 to present day, and from McCarroll and Loader (2004) for the 1890-1958 period.

Average annual values of oxide (NOy) and ammonium (NHx) atmospheric deposition (both dry and wet deposition) for the period from 1850 to 2014 were extracted from the NCAR global data set managed by the IGAC-SPARC CCMI (Chemistry-Climate Model Initiative; available for download at http://blogs.reading.ac.uk/ccmi/)(Figure 2). These N depositions data were generated with the NCAR atmospheric transport model (National Center for Atmospheric Research), which provides gridded (resolution of 2.0°x 2.25°, longitude x latitude) temporal simulations of the chemical composition of the atmosphere.

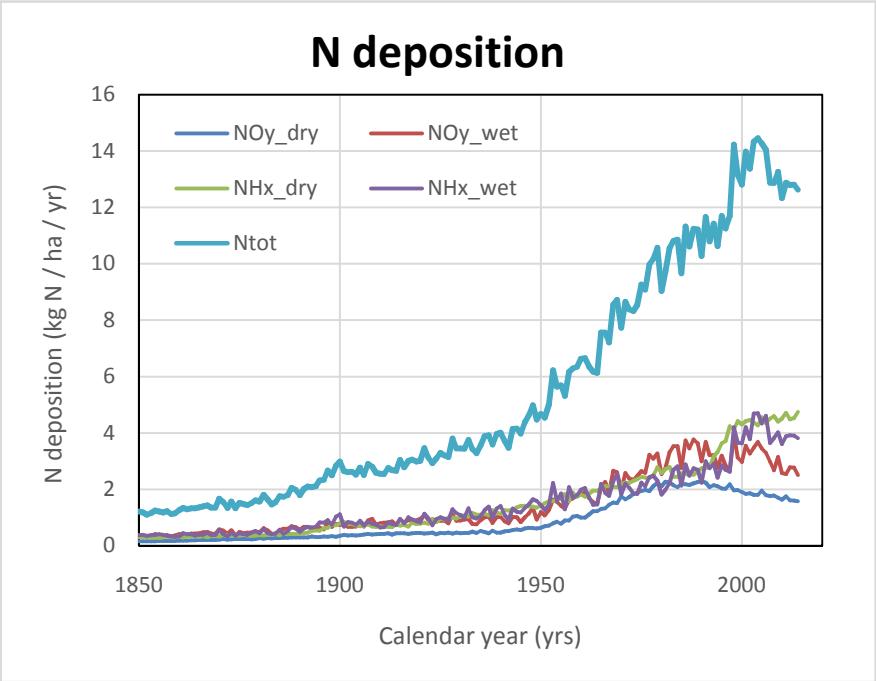
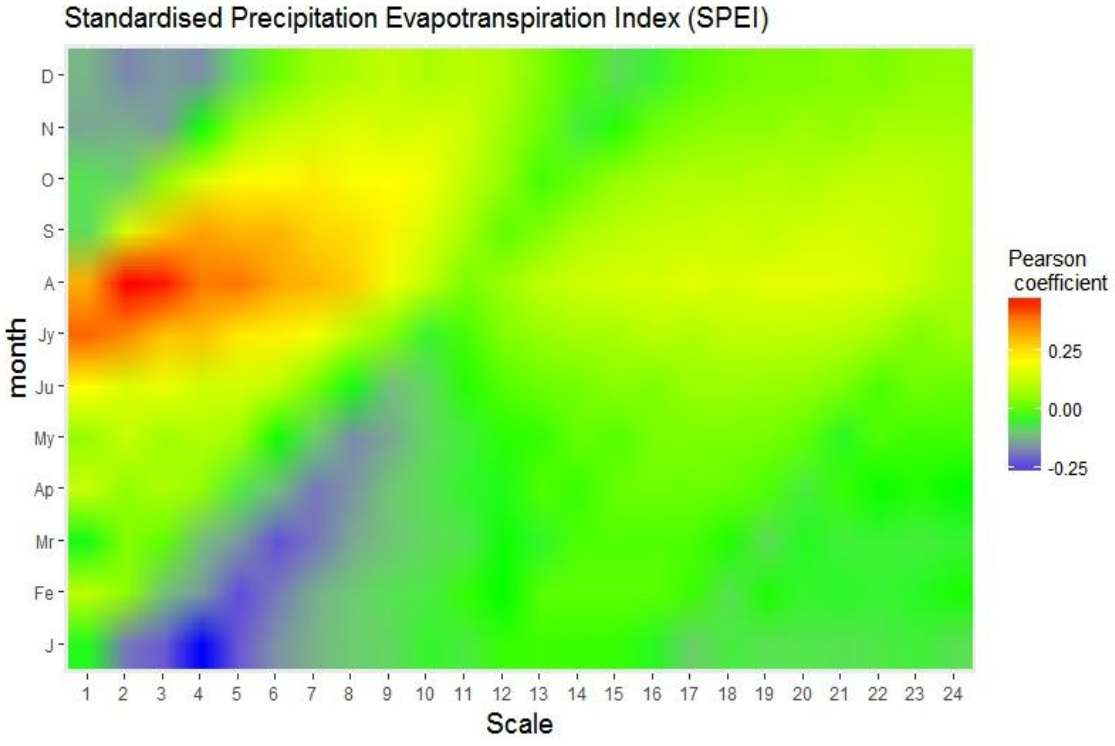


Fig 2. N deposition. Nitrogen deposition trends at Vallombrosa site as modeled by NCAR. Different colors represent the different species (oxide or ammonium) and different form of deposition (wet or dry) plus the total. On the *x*-axis calendar year (yrs), on the *y*-amount of deposition (kg N/ha/yr).

In order to evaluate the potential effects of drought stress, the Standardized Precipitation Evapotranspiration Index (SPEI; Vicente-Serrano et al., 2010) was included in the analysis. SPEI considers the sensitivity to changes in evapotranspiration demand and precipitation (P-PET) at different timescales, computing the cumulate influence of *n* previous months on the water deficit/surplus of the month of interest. Here, P-PET is derived from the Thornthwaite equation (Thornthwaite, 1948). For further calculations, a representative month at defined timescales was selected on the basis of the corresponding Pearson correlation coefficient. Correlations were performed between tree-rings width index series (RWI), de-trended with the negative exponential curve method (most conservative one), and the 1–24 timescale SPEI values computed for each month (Vicente-Serrano et al., 2014; Figure 3-4).



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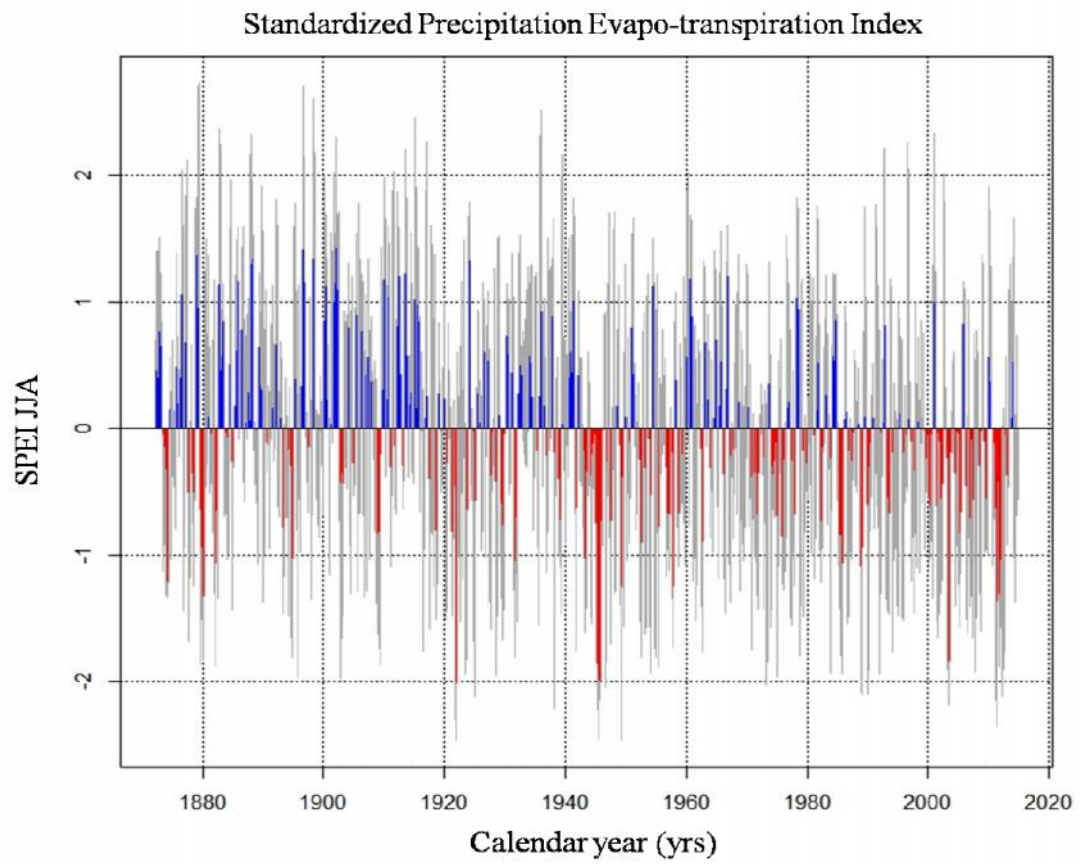
371 **Fig.3 SPEI correlation RWI heatmap**. Correlations (Pearson coefficient) between the Standardized
372 Precipitation Evapotranspiration Index (SPEI) at 1- to 24 month scales, and de-trended tree-rings index
373 (RWI), with on the *x*-axis temporal scale of SPEI and on the *y*-axis related months.



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375 **Fig.4 SPEI JJA**. Trend of August SPEI at 3 month scales (June, July, August), which displays the highest
376 with RWI, with on the *x*-calendar year (yrs) and on the *y*-axis SPEI values centered around 0. Red bar
377 represent water deficit, while blue bars represent water surplus.

2.4 Data analysis

As tree growth exhibits strong non-linear patterns caused by both biological (i.e. age and size) and environmental (i.e. changes in CO2, temperature, precipitation...) drivers, generalized additive models (GAMs; Hastie and Tibshirani 1990) were applied to identify the shape of the inherent relationships existing between BAI and predictor variables. GAMs are non-linear regression models that specify the value of the dependent variable as the sum of smooth functions of independent variables in a non-parametric fashion. Such a model relaxes any *a priori* assumptions of the functional relationship between response and predictors, therefore resulting in a more flexible range of application. It can be expressed as:

$$y_i = \alpha + f_1(x_{i1}) + \cdots + f_n(x_{in}) + \varepsilon_i \qquad \text{for } \varepsilon i \sim N(0,\sigma^2) \tag{2}$$

where y_i is the response variable, α is the unknown intercept of fixed parameters, $x_1,\ldots,x_n$ are independent variables, $f_1,\ldots,fn$ are smooth functions and εi are residuals with normal (Gaussian) distribution and constant variance. The GAM model was applied to log-transformed BAI data, so as to correct for heteroscedasticity. A cubic penalized spline was used as smooth function. This is the result of the simultaneous fitting of basis functions (i.e. natural cubic spline) penalized to achieve the optimal degree of smoothness, avoiding data over-fitting. The amount of penalizations was automatically computed by the maximum likelihood estimation (ML)(Wood, 2017). The selection of covariates was performed by a stepwise backward process. Tree age, atmospheric [CO_2], total atmospheric N deposition or its NH_x and NO_y components, mean (T_m) or maximum (T_{max}) and minimum (T_{min}) annual temperatures, annual precipitation (P) and the SPEI value of the current and previous year ($SPEI_{t-1}$) were considered as possible covariates. Candidates for removal were identified based on their lower approximate p-values and the model resulting after the subtraction of such variables was compared with the previous one based on Bayesan information criterion (BIC). This index was used instead of the Akaike information criterion (AIC) because it is

403 less conservative and more useful to assess the 'true' model in confirmatory analysis; in model
404 selection, the BIC provides a better opportunity to understand which pool of variables represent the
405 simpler model (Aho et al. 2014). All of the GAMs analyses were performed with the mgcv pakage
406 (Wood, 2006) of the R statistical suite (R Core Team, 2017). No pre-whitening processes (i.e
407 addition of an autocorrelation structure of residuals) were applied to the radial increments time
408 series, with the aim to preserve long-term trends. The concurvity level (i.e. the generalization of co-
409 linearity in non-linear models) was also checked to assess a potential correlation among variables
410 (Tab.2). Concurvity could be an issue in models including a time-dependent smooth function with
411 other time-varying covariates, making model estimation unstable (Wood 2006), although GAMs are
412 able to deal with some degree of concurvity (Wood 2008). Finally, model results were tested to
413 ensure that the assumptions of normal distribution of observations and absence of heteroscedasticity
414 of residuals were respected (Fig. 5).

415 **Tab. 2|** Concurvity (collinearity for non-linear regression techniques) between GAM covariates. Values equal to 1
416 represent complete concurvity among covariates. Values under the threshold of 0.5 are deemed acceptable.

Covariate	parameters	Age	CO2	NOy dep	SPEI JJA	SPEI JJA t-1
parameters		1.50E-31	4.62E-32	7.28E-32	1.05E-31	8.60E-33
Age	1.44E-28		1.64E-01	7.88E-02	1.52E-02	1.56E-02
CO2	1.76E-29	1.74E-01		4.61E-01	8.21E-02	8.38E-02
NOy dep	4.88E-30	1.25E-01	5.34E-01		7.17E-02	1.05E-01
SPEI JJA	1.13E-29	1.73E-02	1.03E-01	7.04E-02		6.68E-02
SPEI JJA t-1	3.67E-31	1.84E-02	1.05E-01	1.03E-01	7.20E-02	

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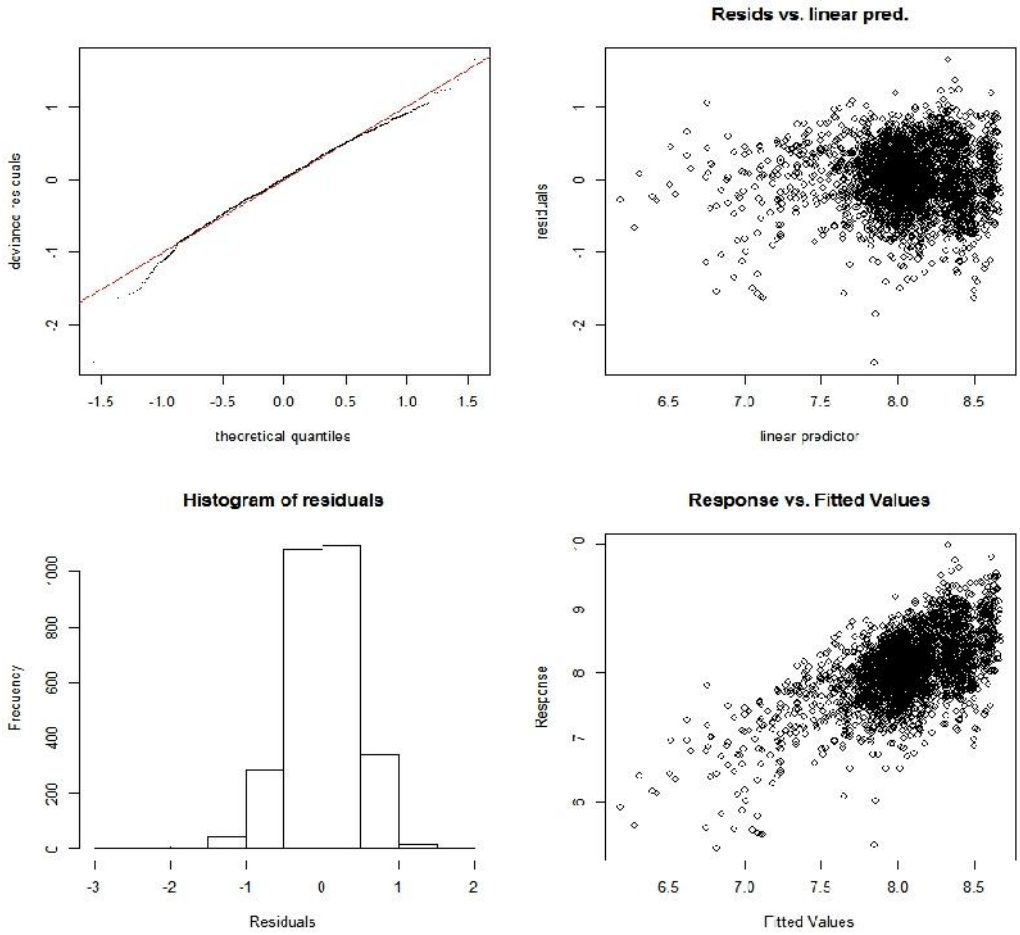


Fig.5 Test of GAMs results for BAI as a function of age and time. Residual distribution of the whole model and against linear predictor. Response against fitted values for the whole model.

3 Results

3.1 Dendrochronology

All of the trees used in this study were satisfactorily cross-dated and no missing rings were detected. The basal area increments of the different age classes are presented in Fig. 6 and Fig. 7, along calendar year and along cambial age respectively. The general statistics of the tree-ring chronologies are summarized in Table 3. The mean series inter-correlation (SI) that represents the strength of the common signal shared by all series is about 0.5, while the expressed population signal is above the conventional threshold (ESP>0.85) used to define the acceptability of the chronology. This index confirms the goodness of cross-dating and the possibility to use this dataset for further analysis. Furthermore, mean sensitivity (MS), which is an index of year-to-year variability related to climate and/or disturbances, was also checked; a value ranking about 0.2

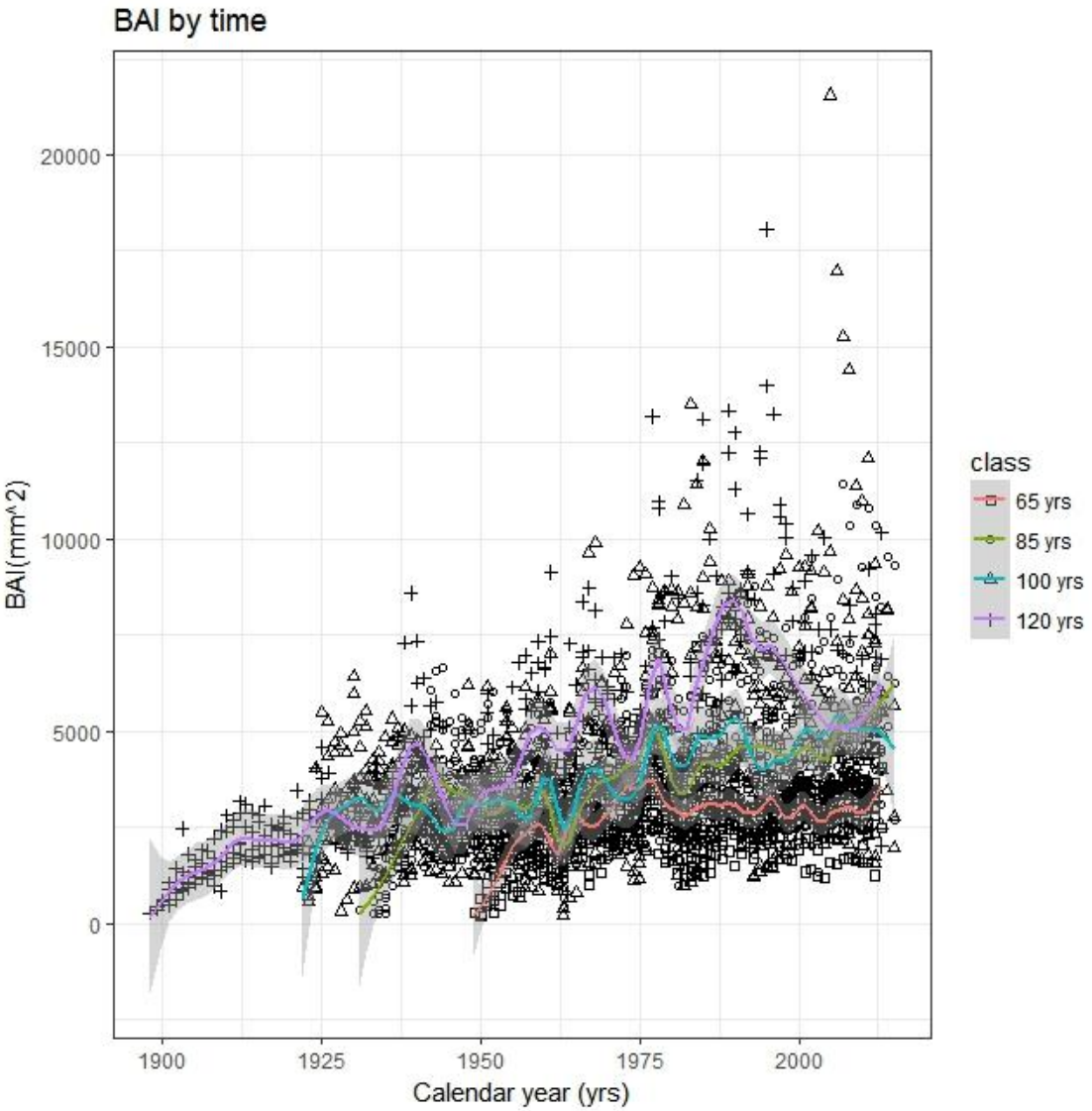
433 shows an adequate sensitive series, normally useful for climatic correlation analysis.

434

Tab.3| Descriptive statistics for raw (TRW) and ring width index (RWI) chronologies of the 4 different age-classes. *MW* is mean ring width, *SD* is standard deviation, *MS* is mean sensitivity, *AR1* the first order autocorrelation, ESP the expressed population signal, *SI* the series inter-correlation

	TRW				RWI	
Age-class	*MW*	*SD*	*MS*	*AR1*	*ESP*	*SI*
70	3.5484	1.6647	0.1488	0.8506		
85	3.4193	2.0175	0.1871	0.855		
100	3.605	1.7718	0.198	0.7519		
120	3.3214	1.2572	0.2004	0.7088		
total	3.495257	1.737886	0.181171	0.8034	**0.855**	**0.50**

435



436

437 **Fig. 6 Time-related dynamics of basal area increments in different age-classes.** Time series of basal area
438 increments (BAI), grouped by age-class and fitted with a cubic spline. The shaded areas indicate the
439 95% prediction interval of the function

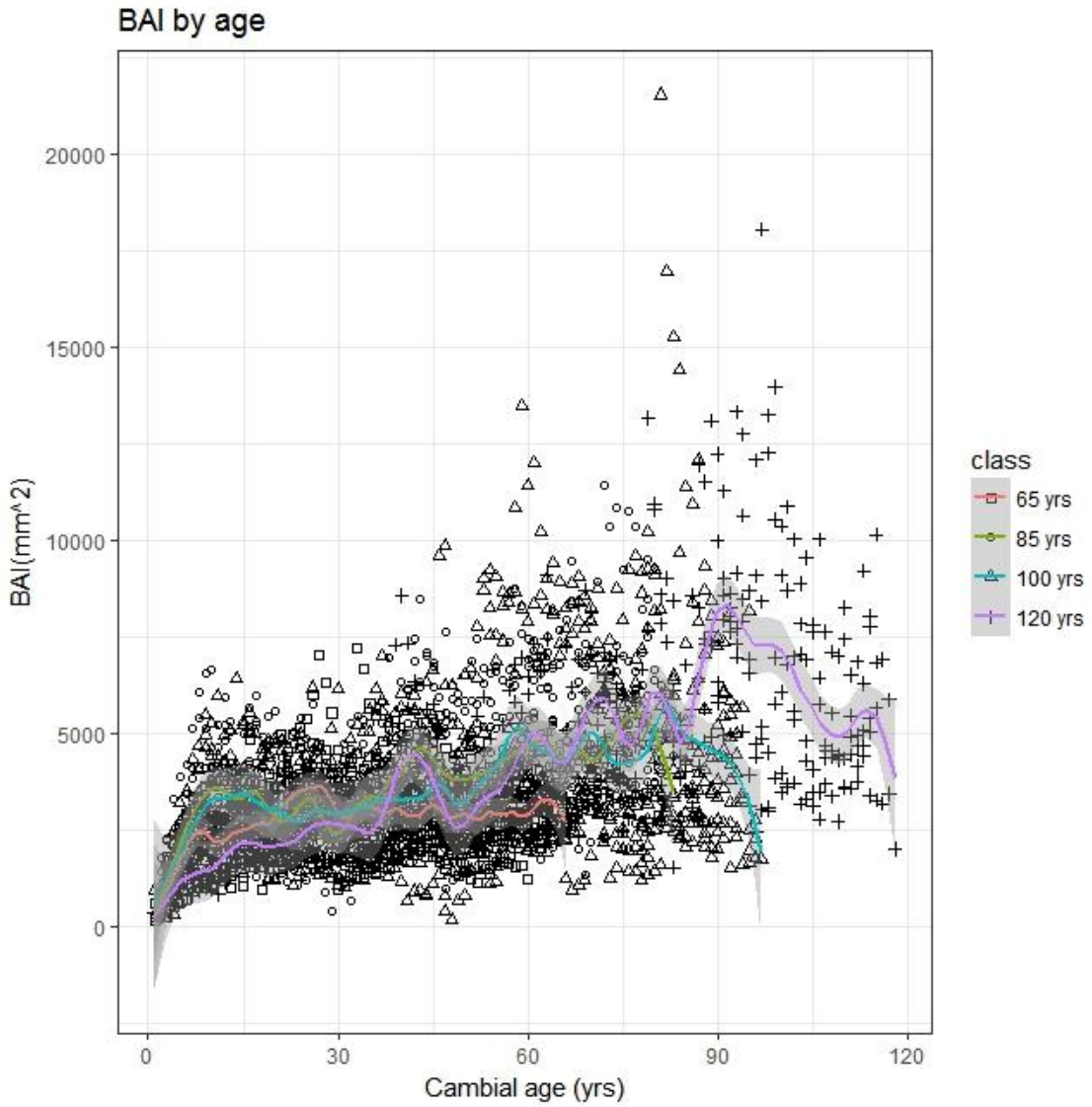


Fig. 7 Diachronic analysis of age effects on basal area increments in different age-classes. Time series of basal area increments (BAI), grouped by age-classes and fitted with a cubic spline. The shaded areas indicate the 95% prediction interval of the spline function

3.2 Model output

In order to assess possible changes of growth rates over time, independent of the co-occurring effects of ontogenetic factors, tree basal area increments (BAI) were modeled as:

$$\ln(\mathrm{BAI}) = f(\mathrm{AGE}) + f(\mathrm{TIME}) + \varepsilon_i \quad (3)$$

where f(AGE) is the cambial age effect and f(TIME) represents all of the environmental effects cumulated into a single global variable, varying over time. The BAI global long-term trend (Fig. 8b), after the subtraction of the age-related signal (Fig. 8a), shows an initial increase, two

culminations around the '30s and the '80s of the last century, a lower growth in between and a subsequent decrease until the first decade of this century. The age-related effect displays the expcted shape, with a steep increase at early age in the first part of the curve, followed by a less pronounced growth, and an apparent culmination at an age of 100.

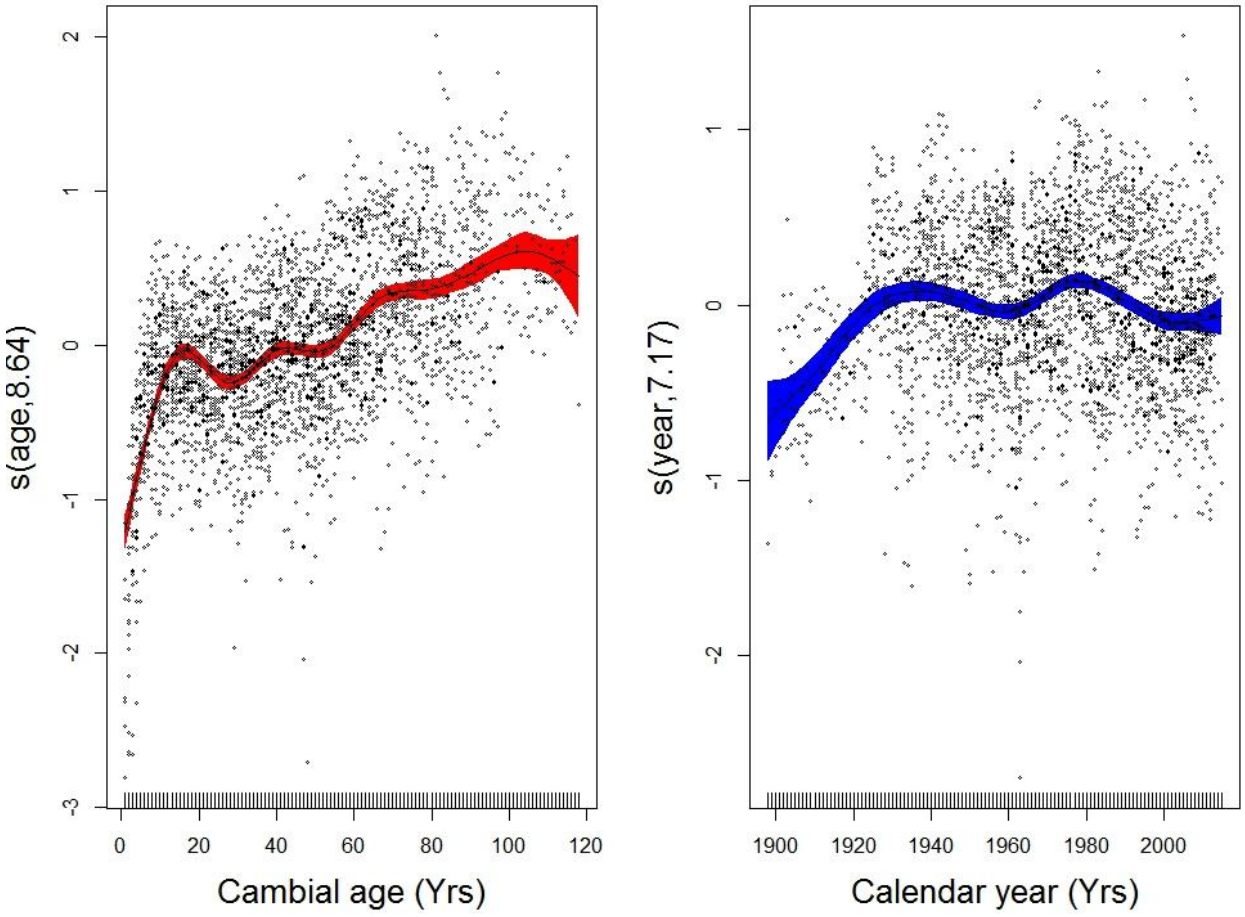


Fig. 8 GAM analysis of the independent effects on BAI of age and time.

a. Trend of basal area increments (BAI) as a function of age , after correcting for time-related effects. On *x*-axis age (years), and on *y*-axis the function of age *f*(Age), dimensionless and centered around 0.

b. Global trend of BAI as a function of time , after correcting for age-related effects. On *y*-axis the function of time *s*(TIME), dimensionless and centered around 0. Points represent partial residuals from the fitted function and the shaded areas indicate the 95% prediction interval of fitted adaptive splines. The GAM model was applied to log-transformed BAI data, so as to correct for heteroscedasticity.

Successively, in order to partition to individual drivers the effect so far attributed to global change, seasonal climatic and geochemical variables were added to the model instead the time variable, and after the backward stepwise variable selection, it was specified as follow:

$$\ln(BAI) = f(\text{Age}) + f(CO_2) + f(NO_{y\ dep}) + f(\text{SPEI JJA}) + f(\text{SPEI JJA t-1}) + \varepsilon_i$$

Age is the age/size effect associated with variations in cambial age, CO_2 is the annual level of atmospheric [CO_2], $NO_{y\ dep}$ is the annual sum of dry and wet deposition of oxide N (NO_y) species, SPEI JJA and SPEI JJA t-1 represent the summer SPEI (Standardized Precipitation-Evapotranspiration Index; Vicente-Serrano et al. 2010) values in the ongoing and previous summer, respectively. All variables exhibit a significant p-value at 0.001 level (Tab. 4), with a global adjusted R^2 for the whole model of 0.371.

Tab. 4| Generalized additive model (GAM) results. Climatic and biological factors relationships with BAI series (as a dependent variable) in *Pseudotsuga menziesii*. *e.d.f.* are effective degrees of freedom, *F* is the F-test for variance explained, *P* is the p-values and $R^2(adj)$ is the adjusted regression coefficient of the entire model.

Factor	*e.d.f.*	*F*	*P*	$R^2(adj)$
Age	8.652	83.872	< 2e-16	
CO2	7.172	14.951	< 2e-16	
Noy dep	3.485	1.614	0.000705	
SPEI JJA	1.961	3.513	4.80E-09	
SPEI JJA t-1	1.757	2.16	5.25E-06	
Whole model				0.371

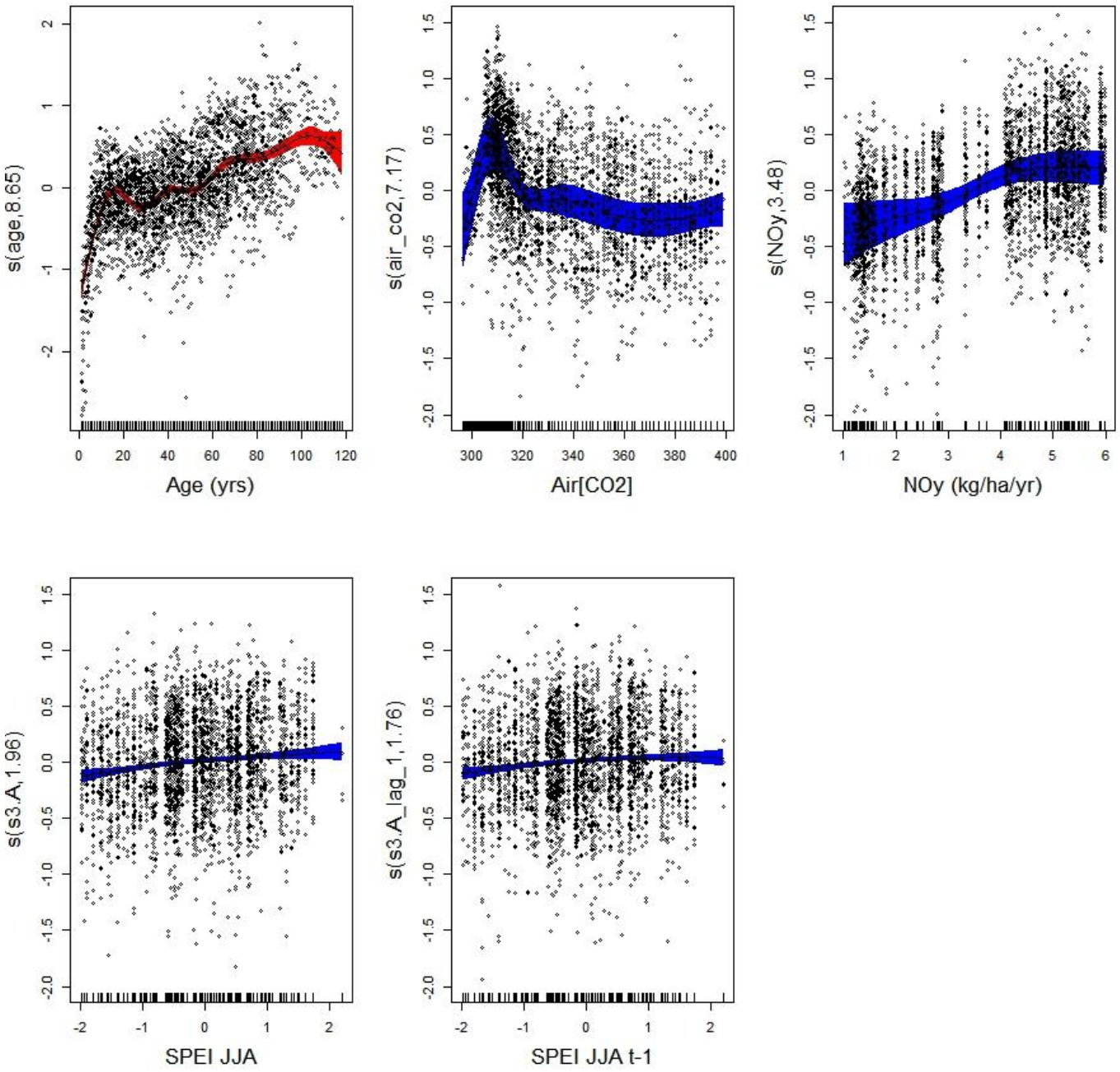


Fig. 9. GAM analysis of increment response to individual drivers. Generalized additive models (GAMs) results show the relationship between basal area increments (BAI) and environmental and biological factors remaining after the backward selection procedure: cambial age, atmospheric [CO_2] and Standardized Precipitation Evapotranspiration Index computed over June , July and August of the current year (SPEI JJA) and of the previous year (SPEI JJA_{t-1}) . Values on the *y*-axis indicate the independent effect of each covariate on basal area increments, as predicted by the model (continuous line) dimensionless and centered around 0, plus the estimated degree of freedom (edf). Points represent partial residuals from the fitted function and the shaded areas indicate the 95% prediction interval. The GAM model was applied to log-transformed BAI data, so as to correct for heteroscedasticity.

4. Discussion

The primary purpose of this study was to assess if any changes in growth rates have occurred over time in Douglas-fir in the northern Apennines, once correcting for age-related patterns. The global long-term trend illustrates a decrease in the productivity of this species in the last four decades, amounting to about 22.9%. These findings appear to be consistent with several other studies that looked at forest growth changes in central Apennines (Piovesan et al. 2008), in the Mediterranean region by and large (Linares et al.,2010) and in other European areas (Vitas and Žeimavičius 2006). All of these studies found that the increase in summer drought had a negative effect on growth, in association with co-varying factors, such as stand dynamics, competition and/or pests. These could exacerbate the role of the imbalance in water availability and overcome the potentially positive effects of atmospheric nitrogen deposition, of the increase in the length of the growing season, and of the rise in atmosphere [CO_2]. The general trend recorded in this study can be partially explained by examining the shape of the relationship between significant environmental factors and BAI. The response to atmospheric [CO_2] (Fig. 9b) presents a strong positive pattern at the low end of the concentration range, with a culmination at around 310 ppm, followed by a decline and an apparent lack of effect at higher concentrations. Although the lack of evidence of a clear fertilization effect of CO_2 is in agreement with previous studies (Peñuelas et al. 2011; Lévesque et al. 2014), it could lead to different conclusions, depending on the processes involved. In a biological perspective, for example, both long-term photosynthetic acclimation (Medlyn et al. 1999) and a shift in allocation of assimilated C to faster-turnover pools such as fine roots or canopy foliage (Korner et al., 2005) are possible explanations. Moreover, this lack of response could be the result of an interaction between CO_2 and nutrient availability effects, which cannot be accounted by a simple additive model. Finally, such a apparent saturation effect of CO_2 could be the result of the lack of significant variables, not included in the model, as for example inter-tree competition. Indeed the radial growth of shade intolerant trees is very much dependent on forest management practices, especially in even-aged stands. Moreover, the extent of drought events could have been insufficiently

represented by the rather crude approach applied in the study. Summer water availability (Figure 9d), which is related to the transpiration demand, is the second most important variable affecting the behavior of this species in the long-term, conditioning its growth performance (Beedlow et al. 2013) and distribution (Rehfeldt et al. 2014). Air dryness, which is also known to affect Douglas fir, was not included as a potential driver due to a lack of suitable information. Furthermore, the influence of the previous growing seasons' summer water balance (Figure 9e) also affects the growth trend, as early-wood width is related to the amount of carbon storage reserves built-up in the preceding year, which are subject to remobilisation in the first phase of vegetative growth (Lee et al. 2016). At last, our findings suggest a positive relationship between growth and N deposition (Figure 9c), which potentially reflects the beneficial effect of N increase on photosynthetic rates due to the resulting increase in photosynthetic pigments as well as Rubisco foliar content. The possible stabilization observed at the higher rate of N deposition, if significant, could be interpreted as a saturation of the nitrogen effect on the system. Although N-mineralisation rates at the site are not known, such a saturation above a deposition a N deposition rate of 4.5 kg /ha/yr, however, seems unlikely since Douglas-fir soils at the site display rather high C:N ratios, with an average value of 27 (Di Biase et al. 2015), although N mineralisation data are not available to support such the hypothesis of substantial N limitations. Besides, N uptake by Douglas-fir was found to increase asymptotically, until at least 35 kg N ha^{-1} yr^{-1} of net nitrogen available (Perakis and Sinkhorn 2011) in US Pacific Coast environments. A possible influence caused by concurvity with other factors, namely CO_2 concentration, should be also taken into consideration. Indeed, when non-stationary forcing factors (i.e., atmospheric [CO_2] and nitrogen deposition) co-vary, it is difficult to disentangle their individual effects on long-term tree growth, and this complication increases with the complexity of the model (Carrer and Urbinati 2006).

5 Conclusions

Given the importance of Douglas-fir as a timber species, the ongoing decrease in growth performance illustrated by this study for the northern Apennines could have relevant implications

from a management perspective. For this reason, understanding which factors have been determining such a trend is particularly important. Our model, despite the rather low amount of variance explained and the simplicity of the model structure (only few variables considered), as well as its additive nature, allows us to draw some conclusions. The impact of summer water availability, which is projected to decrease in the Mediterranean region (IPCC, 2014), could be responsible to a considerable extent for the observed decrease in growth rates in recent decades, due to the increase in magnitude and frequency of drought events. A parallel positive effect ascribable to N deposition, which should have promoted the stem growth in the past, may no longer be able to counterbalance the summer drought stress effect, due to the stabilization in NOy emission and an apparent saturation of the N response. Especially in the absence of a positive effect of fertilization by rising atmospheric [CO_2], the observed trend can be expected to be exacerbated in the next future.

Finally, GAMs appear to have a promising potential to disentangle non-linear biological and environmental effects affecting tree growth, resulting in long-term trend preservation, which is fundamental if a better understanding of past environmental effects is to be used to understand the future behavior of forests in a changing world.

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7. Bibliography

ABER, JOHN D., CHRISTINE L. GOODALE, SCOTT V. OLLINGER, MARIE-LOUISE SMITH, ALISON H. MAGILL, MARY E. MARTIN, RICHARD A. HALLETT, and JOHN L. STODDARD. 2003. “Is Nitrogen Deposition Altering the Nitrogen Status of Northeastern Forests?” *BioScience* 53 (4): 375. doi:10.1641/0006-3568(2003)053[0375:INDATN]2.0.CO;2.

Aho, Ken, DeWayne Derryberry, and Teri Peterson. 2014. “Model Selection for Ecologists: The Worldview of AIC and BIC.” *Ecology* 95 (March): 631–36. doi:10.1890/13-1452.1.

572 Ainsworth, Elizabeth A., and Stephen P. Long. 2005. “What Have We Learned from 15 Years of
573 Free-Air CO2 Enrichment (FACE)? A Meta-Analytic Review of the Responses of
574 Photosynthesis, Canopy Properties and Plant Production to Rising CO2.” *New Phytologist* 165
575 (2): 351–71. doi:10.1111/j.1469-8137.2004.01224.x.

576 Babst, Flurin, M. Ross Alexander, Paul Szejner, Olivier Bouriaud, Stefan Klesse, John Roden,
577 Philippe Ciais, et al. 2014. “A Tree-Ring Perspective on the Terrestrial Carbon Cycle.”
578 *Oecologia* 176 (2): 307–22. doi:10.1007/s00442-014-3031-6.

579 Beedlow, Peter A., E. Henry Lee, David T. Tingey, Ronald S. Waschmann, and Connie A. Burdick.
580 2013. “The Importance of Seasonal Temperature and Moisture Patterns on Growth of Douglas-
581 Fir in Western Oregon, USA.” *Agricultural and Forest Meteorology* 169. Elsevier B.V.: 174–
582 85. doi:10.1016/j.agrformet.2012.10.010.

583 Biondi, Franco. 1999. “Comparing Tree-Ring Chronologies and Repeated timberiInventories as
584 Forest Monitoring Tools.” *Ecological Applications* 9 (1): 216–27. doi:10.1890/1051-
585 0761(1999)009[0216:CTRCAR]2.0.CO;2.

586 Boettger, Tatjana, Marika Haupt, Kay Knöller, Stephan M. Weise, John S. Waterhouse, Katja T.
587 Rinne, Neil J. Loader, et al. 2007. “Wood Cellulose Preparation Methods and Mass
588 Spectrometric Analyses of ??13C, ??18O, and Nonexchangeable ??2H Values in Cellulose,
589 Sugar, and Starch: An Interlaboratory Comparison.” *Analytical Chemistry* 79 (12): 4603–12.
590 doi:10.1021/ac0700023.

591 Boisvenue, Céline, and Steven W. Running. 2006. “Impacts of Climate Change on Natural Forest
592 Productivity - Evidence since the Middle of the 20th Century.” *Global Change Biology* 12 (5):
593 862–82. doi:10.1111/j.1365-2486.2006.01134.x.

594 Brienen, R J W, E Gloor, S Clerici, R Newton, L Arppe, A Boom, S Bottrell, et al. n.d. “Isotopes.”
595 *Nature Communications*. Springer US, 1–10. doi:10.1038/s41467-017-00225-z.

596 Brunel, J.P., G.R. Walker, C.D. Walker, J.C. Dighton, and A. Kennett-Smith. 1991. “Using Stable
597 Isotopes of Water to Trace Plant Water Uptake.” *International Symposium on the Use of Stable
598 Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies*, 543–551.
599 doi:10.2144/000114133.

600 Bunn, Andrew G. 2008. “A Dendrochronology Program Library in R (dplR).” *Dendrochronologia*
601 26 (2): 115–24. doi:10.1016/j.dendro.2008.01.002.

602 Camarero, J. Julio, Antonio Gazol, Jacques C. Tardif, and France Conciatori. 2015. “Attributing
603 Forest Responses to Global-Change Drivers: Limited Evidence of a CO2-Fertilization Effect in
604 Iberian Pine Growth.” *Journal of Biogeography* 42 (11): 2220–33. doi:10.1111/jbi.12590.

605 Carrer, Marco, and C Urbinati. 2006. “Long-Term Change in the Sensitivity of Tree- ring Growth
606 to Climate Forcing in *Larix Decidua*.” *New Phytologist* 170 (iv): 861–72.

607 Cook, Edward R., Keith R. Briffa, David M Meko, Donald A Graybill, and Gary Funkhouser. 1995.
608 “The ’segment Length Curse’ in Long Tree-Ring Chronology Development for Palaeoclimatic
609 Studies.” *The Holocene* 5 (2): 229–37. doi:10.1177/095968369500500211.

610 Dawson, T E. 1998. “ Fog in the Californian Redwood Forest: Ecosystem Inputs and Use by
611 Plants.” *Oecologia* 117: 476–85.

612 Dawson, Todd E., Stefania Mambelli, Agneta H. Plamboeck, Pamela H. Templer, and Kevin P. Tu.

2002. “Stable Isotopes in Plant Ecology.” *Annual Review of Ecology and Systematics* 33 (1): 507–59. doi:10.1146/annurev.ecolsys.33.020602.095451.

Dawson, Todd E., and John S Pate. 1996. “Seasonal Water Uptake and Movement in Root Systems of Australian Phraeatophytic Plants of Dimorphic Root Morphology: A Stable Isotope Investigation.” *Oecologia* 107 (1): 13–20. doi:10.1007/BF00582230.

Di Biase, Giampaolo, Gloria Falsone, Anna Graziani, Gilmo Vianello, and Livia Vittori Antisari. 2015. “Carbon Sequestration in Soils Affected By Douglas Fir Reforestation in Apennines (Northern Italy).” *Eqa-International Journal of Environmental Quality* 17: 1–11. doi:10.6092/issn.2281-4485/5208.

Dongmann, G, and H W Nürnberg. 1974. “On the Enrichment of H2180 in the Leaves of Transpiring Plants I T L Q E” 52: 1–2.

Esper, Jan, Edward R Cook, and Fritz H Schweingruber. 2002. “Low-Frequency Signals in Long Tree-Ring Chronologies for Reconstructing Past Temperature Variability.” *Science (New York, N.Y.)* 295 (5563). American Association for the Advancement of Science: 2250–53. doi:10.1126/science.1066208.

Esper, Jan, David C. Frank, Giovanna Battipaglia, Ulf Büntgen, Christopher Holert, Kerstin Treydte, Rolf Siegwolf, and Matthias Saurer. 2010a. “Low-Frequency Noise in δ13C and δ18O Tree Ring Data: A Case Study of Pinus Uncinata in the Spanish Pyrenees.” *Global Biogeochemical Cycles* 24 (4): 1–11. doi:10.1029/2010GB003772.

Esper, Jan, David C Frank, Giovanna Battipaglia, Ulf Büntgen, Christopher Holert, Kerstin Treydte, Rolf Siegwolf, and Matthias Saurer. 2010b. “Low - Frequency Noise in D 13 C and D 18 O Tree Ring Data : A Case Study of Pinus Uncinata in the Spanish Pyrenees” 24 (2): 1–11. doi:10.1029/2010GB003772.

Farquhar, G. D., L. A. Cernusak, and B. Barnes. 2006. “Heavy Water Fractionation during Transpiration.” *Plant Physiology* 143 (1): 11–18. doi:10.1104/pp.106.093278.

Farquhar, G D, J R Ehleringer, and K T Hubick. 1989. “Carbon Isotope Discrimination and Photosynthesis.” *Annual Review of Plant Physiology and Plant Molecular Biology*. doi:10.1146/annurev.pp.40.060189.002443.

Federal, Swiss, Universitat De Barcelona, J. Julio Camarero, Antonio Gazol, Juan Diego Galván, Gabriel Sangüesa-Barreda, and Emilia Gutiérrez. 2015. “Disparate Effects of Global-Change Drivers on Mountain Conifer Forests: Warming-Induced Growth Enhancement in Young Trees vs. CO 2 Fertilization in Old Trees from Wet Sites.” *Global Change Biology* 21 (2): 738–49. doi:10.1111/gcb.12787.

Fenn, Mark E, Jeremy S Fried, Haiganoush K Preisler, Andrzej Bytnerowicz, Susan Schilling, Sarah Jovan, and Olaf Kuegler. 2015. “REMEASURED FIA PLOTS REVEAL TREE-LEVEL DIAMETER GROWTH AND TREE MORTALITY IMPACTS OF NITROGEN DEPOSITION ON CALIFORNIA ’ S FORESTS” 2013 (Time 2): 2013–16.

Francey, R. J., C. E. Allison, D. M. Etheridge, C. M. Trudinger, I. G. Enting, M. Leuenberger, R. L. Langenfelds, E. Michel, and L. P. Steele. 1999. “A 1000-Year High Precision Record of δ13C in Atmospheric CO2.” *Tellus, Series B: Chemical and Physical Meteorology* 51 (2): 170–93. doi:10.1034/j.1600-0889.1999.t01-1-00005.x.

Frank, D. C., B. Poulter, M. Saurer, J. Esper, C. Huntingford, G. Helle, K. Treydte, et al. 2015.

"Water-Use Efficiency and Transpiration across European Forests during the Anthropocene." *Nature Climate Change*, no. May. doi:10.1038/nclimate2614.

Giustini, Francesca, Mauro Brilli, and Antonio Patera. 2016. "Mapping Oxygen Stable Isotopes of Precipitation in Italy." *Journal of Hydrology: Regional Studies* 8. Elsevier B.V.: 162–81. doi:10.1016/j.ejrh.2016.04.001.

Gómez-Guerrero, Armando, Lucas C.R. R Silva, Miguel Barrera-Reyes, Barbara Kishchuk, Alejandro Velázquez-Martínez, Tomás Martínez-Trinidad, Francisca Ofelia Plascencia-Escalante, and William R. Horwath. 2013. "Growth Decline and Divergent Tree Ring Isotopic Composition (δ13C and δ18O) Contradict Predictions of CO2 Stimulation in High Altitudinal Forests." *Global Change Biology* 19 (6): 1748–58. doi:10.1111/gcb.12170.

Griffin, Daniel, and Kevin J Anchukaitis. 2014. "How Unusual Is the 2012 – 2014 California Drought ?," 9017–23. doi:10.1002/2014GL062433.1.

Guerrieri, R, Maurizio Mencuccini, L J Sheppard, M Saurer, M P Perks, P Levy, M a Sutton, Marco Borghetti, and J Grace. 2011. "The Legacy of Enhanced N and S Deposition as Revealed by the Combined Analysis of Delta 13C, Delta 18O and Delta 15N in Tree Rings." *Global Change Biology* 17 (5): 1946–62. doi:10.1111/j.1365-2486.2010.02362.x.

Harris, I., P. D. Jones, T. J. Osborn, and D. H. Lister. 2014. "Updated High-Resolution Grids of Monthly Climatic Observations - the CRU TS3.10 Dataset." *International Journal of Climatology* 34 (3): 623–42. doi:10.1002/joc.3711.

Hastie, T. J., and R. J. Tibshirani. 1990. "Generalized Additive Models." *Monographs on Statistics and Applied Probability*. doi:10.1016/j.csda.2010.05.004.

IPCC. 2014. "Climate Change 2014 Synthesis Report Summary Chapter for Policymakers." *Ipcc*, 31. doi:10.1017/CBO9781107415324.

Keenan, Trevor F, David Y Hollinger, Gil Bohrer, Danilo Dragoni, J William Munger, Hans Peter Schmid, and Andrew D Richardson. 2013. "Increase in Forest Water-Use Efficiency as Atmospheric Carbon Dioxide Concentrations Rise." *Nature* 499 (7458): 324–27. doi:10.1038/nature12291.

Korner, C. 2005. "Carbon Flux and Growth in Mature Deciduous Forest Trees Exposed to Elevated CO2." *Science* 309 (5739): 1360–62. doi:10.1126/science.1113977.

Leavitt, Steven W. 2010. "Tree-Ring C-H-O Isotope Variability and Sampling." *The Science of the Total Environment* 408 (22): 5244–53. doi:10.1016/j.scitotenv.2010.07.057.

Lee, E. Henry, Peter A. Beedlow, Ronald S. Waschmann, David T. Tingey, Charlotte Wickham, Steve Cline, Michael Bollman, and Cailie Carlile. 2016. "Douglas-Fir Displays a Range of Growth Responses to Temperature, Water, and Swiss Needle Cast in Western Oregon, USA." *Agricultural and Forest Meteorology* 221. Elsevier B.V.: 176–88. doi:10.1016/j.agrformet.2016.02.009.

Leonardi, Stefano, Tiziana Gentilesca, Rossella Guerrieri, Francesco Ripullone, Federico Magnani, Maurizio Mencuccini, Twan V. Noije, and Marco Borghetti. 2012. "Assessing the Effects of Nitrogen Deposition and Climate on Carbon Isotope Discrimination and Intrinsic Water-Use Efficiency of Angiosperm and Conifer Trees under Rising CO 2 Conditions." *Global Change Biology* 18 (9): 2925–44. doi:10.1111/j.1365-2486.2012.02757.x.

696 Lévesque, Mathieu, Rolf Siegwolf, Matthias Saurer, Britta Eilmann, and Andreas Rigling. 2014.
697 "Increased Water-Use Efficiency Does Not Lead to Enhanced Tree Growth under Xeric and
698 Mesic Conditions." *New Phytologist* 203 (1): 94–109. doi:10.1111/nph.12772.

699 Linares, Juan Carlos, Jesús Julio Camarero, and José Antonio Carreira. 2010. "Competition
700 Modulates the Adaptation Capacity of Forests to Climatic Stress: Insights from Recent Growth
701 Decline and Death in Relict Stands of the Mediterranean Fir Abies Pinsapo." *Journal of*
702 *Ecology* 98 (3): 592–603. doi:10.1111/j.1365-2745.2010.01645.x.

703 Magnani, Federico, Maurizio Mencuccini, Marco Borghetti, Paul Berbigier, Frank Berninger,
704 Sylvain Delzon, Achim Grelle, et al. 2007. "The Human Footprint in the Carbon Cycle of
705 Temperate and Boreal Forests." *Nature* 447 (7146): 848–50. doi:10.1038/nature05847.

706 Marshall, D D, and R O Curtis. 2002. "Levels-of-Growing-Stock Cooperative Study in Douglas-
707 Fir: Report No. 15 - Hoskins: 1963-1998." *Research Paper Pacific Northwest Research*
708 *Station, USDA Forest Service*, no. PNW-RP-537: 80.

709 McCarroll, Danny, and Neil J. Loader. 2004. "Stable Isotopes in Tree Rings." *Quaternary Science*
710 *Reviews* 23 (7–8): 771–801. doi:10.1016/j.quascirev.2003.06.017.

711 McMahon, Sean M, Geoffrey G Parker, and Dawn R Miller. 2010. "Evidence for a Recent Increase
712 in Forest Growth." *Proceedings of the National Academy of Sciences of the United States of*
713 *America* 107 (8): 3611–15. doi:10.1073/pnas.0912376107.

714 Medlyn, B. E., F. -W. Badeck, D. G. G. De Pury, C. V. M. Barton, M. Broadmeadow, R.
715 Ceulemans, P. De Angelis, et al. 1999. "Effects of Elevated [CO2] on Photosynthesis in
716 European Forest Species: A Meta-Analysis of Model Parameters." *Plant, Cell & Environment*
717 22 (12): 1475–1495. doi:10.1046/j.1365-3040.1999.00523.x.

718 Nair, Richard K F, Micheal P Perks, Andrew Weatherall, Elizabeth M Baggs, and Maurizio
719 Mencuccini. 2015. "Does Canopy Nitrogen Uptake Enhance Carbon Sequestration by Trees?"
720 *Global Change Biology*, September. doi:10.1111/gcb.13096.

721 Nehrbass-Ahles, Christoph, Flurin Babst, Stefan Klesse, Magdalena Nötzli, Olivier Bouriaud,
722 Raphael Neukom, Matthias Dobbertin, and David Frank. 2014. "The Influence of Sampling
723 Design on Tree-Ring-Based Quantification of Forest Growth." *Global Change Biology* 20 (9).
724 doi:10.1111/gcb.12599.

725 Norby, Richard J, Jeffrey M Warren, Colleen M Iversen, Belinda E Medlyn, and Ross E
726 McMurtrie. 2010. "CO2 Enhancement of Forest Productivity Constrained by Limited Nitrogen
727 Availability." *Proceedings of the National Academy of Sciences of the United States of*
728 *America* 107 (45): 19368–73. doi:10.1073/pnas.1006463107.

729 Peñuelas, Josep, Josep G. Canadell, and Romà Ogaya. 2011. "Increased Water-Use Efficiency
730 during the 20th Century Did Not Translate into Enhanced Tree Growth." *Global Ecology and*
731 *Biogeography* 20 (4): 597–608. doi:10.1111/j.1466-8238.2010.00608.x.

732 Perakis, Steven S., and Emily R. Sinkhorn. 2011. "Biogeochemistry of a Temperate Forest Nitrogen
733 Gradient." *Ecology* 92 (7): 1481–91. doi:10.1890/10-1642.1.

734 Peters, Richard L., Peter Groenendijk, Mart Vlam, and Pieter a. Zuidema. 2015. "Detecting Long-
735 Term Growth Trends Using Tree Rings: A Critical Evaluation of Methods." *Global Change*
736 *Biology*, n/a-n/a. doi:10.1111/gcb.12826.

737 Phillips, Nathan G., Thomas N. Buckley, and David T. Tissue. 2008. “Capacity of Old Trees to
738 Respond to Environmental Change.” *Journal of Integrative Plant Biology* 50 (11): 1355–64.
739 doi:10.1111/j.1744-7909.2008.00746.x.

740 Piovesan, Gianluca, Franco Biondi, Aalfredo Di Filippo, Alfredo Alessandrini, and Maurizio
741 Maugeri. 2008. “Drought-Driven Growth Reduction in Old Beech (Fagus Sylvatica L.) Forests
742 of the Central Apennines, Italy.” *Global Change Biology* 14 (6): 1265–81. doi:10.1111/j.1365-
743 2486.2008.01570.x.

744 Poage, Nathan J, and John C Tappeiner, II. 2002. “Long-Term Patterns of Diameter and Basal Area
745 Growth of Old-Growth Douglas-Fir Trees in Western Oregon.” *Canadian Journal of Forest*
746 *Research* 32: 1232–43. doi:10.1139/x02-045.

747 Rehfeldt, Gerald E., Barry C. Jaquish, Javier Lòpez-Upton, Cuauhtémocmoc Sáenz-Romero, J.
748 Bradley St Clair, Laura P. Leites, and Dennis G. Joyce. 2014. “Comparative Genetic
749 Responses to Climate for the Varieties of Pinus Ponderosa and Pseudotsuga Menziesii:
750 Realized Climate Niches.” *Forest Ecology and Management* 324. Elsevier B.V.: 126–37.
751 doi:10.1016/j.foreco.2014.02.035.

752 Ripullone, Francesco, Marco Lauteri, Giacomo Grassi, Mariana Amato, and Marco Borghetti. 2004.
753 “Variation in Nitrogen Supply Changes Water-Use Efficiency of Pseudotsuga Menziesii and
754 Populus X Euroamericana; a Comparison of Three Approaches to Determine Water-Use
755 Efficiency.” *Tree Physiology* 24 (6): 671–79.

756 Rita, Angelo, Marco Borghetti, Luigi Todaro, and Antonio Saracino. 2016. “Interpreting the
757 Climatic Effects on Xylem Functional Traits in Two Mediterranean Oak Species: The Role of
758 Extreme Climatic Events.” *Frontiers in Plant Science* 7 (August): 1–11.
759 doi:10.3389/fpls.2016.01126.

760 Roden, John, and Rolf Siegwolf. 2012. “Is the Dual-Isotope Conceptual Model Fully Operational?”
761 *Tree Physiology* 32 (10): 1179–82. doi:10.1093/treephys/tps099.

762 Rozanski, Kazimierz, Luis Araguas-araguas, and Roberto Gonfiantini. 2016. “Relation Between
763 Long-Term Trends of Oxygen-18 Isotope Composition of Precipitation and Climate Author (S
764): Kazimierz Rozanski , Luis Araguás-Araguás and Roberto Gonfiantini Published by :
765 American Association for the Advancement of Science Stable URL :” 258 (5084): 981–85.

766 Ryan, Mg, D Binkley, and Jh Fownes. 1997. “Age-Related Decline in Forest Productivity: Pattern
767 and Process.” *Advances in Ecological Research*.

768 Ryan, Michael G., Nathan Phillips, and Barbara J. Bond. 2006. “The Hydraulic Limitation
769 Hypothesis Revisited.” *Plant, Cell and Environment* 29 (3): 367–81. doi:10.1111/j.1365-
770 3040.2005.01478.x.

771 Ryan, Michael G., and Barbara J. Yoder. 1997. “Hydraulic Limits to Tree Height and Tree
772 Growth.” *BioScience* 47 (4): 235–42. doi:10.2307/1313077.

773 Saurer, Matthias, Rolf T. W. Siegwolf, and Fritz H. Schweingruber. 2004. “Carbon Isotope
774 Discrimination Indicates Improving Water-Use Efficiency of Trees in Northern Eurasia over
775 the Last 100 Years.” *Global Change Biology* 10 (12): 2109–20. doi:10.1111/j.1365-
776 2486.2004.00869.x.

777 Scheidegger, Y., M. Saurer, M. Bahn, and R. Siegwolf. 2000. “Linking Stable Oxygen and Carbon
778 Isotopes with Stomatal Conductance and Photosynthetic Capacity: A Conceptual Model.”

779 *Oecologia* 125 (3): 350–57. doi:10.1007/s004420000466.

780 Sitch, Stephan, C. Huntingford, N. Gedney, P. E. Levy, M. Lomas, S. L. Piao, R. Betts, et al. 2008.
781 "Evaluation of the Terrestrial Carbon Cycle, Future Plant Geography and Climate-Carbon
782 Cycle Feedbacks Using Five Dynamic Global Vegetation Models (DGVMs)." *Global Change*
783 *Biology* 14 (9): 2015–39. doi:10.1111/j.1365-2486.2008.01626.x.

784 Sternberg, Leonel Da Silveira Lobo O.Reilly. 2009. "Oxygen Stable Isotope Ratios of Tree-Ring
785 Cellulose: The next Phase of Understanding." *New Phytologist* 181 (3): 553–62.
786 doi:10.1111/j.1469-8137.2008.02661.x.

787 Treydte, Kerstin, Sonja Boda, Elisabeth Graf Pannatier, Patrick Fonti, David Frank, Bastian Ullrich,
788 Matthias Saurer, et al. 2014. "Seasonal Transfer of Oxygen Isotopes from Precipitation and
789 Soil to the Tree Ring : Source Water versus Needle Water Enrichment."

790 Vicente-Serrano, Sergio M., Santiago Beguería, and Juan I. López-Moreno. 2010. "A Multiscalar
791 Drought Index Sensitive to Global Warming: The Standardized Precipitation
792 Evapotranspiration Index." *Journal of Climate* 23 (7): 1696–1718.
793 doi:10.1175/2009JCLI2909.1.

794 Vicente-Serrano, Sergio M., J. Julio Camarero, and Cesar Azorin-Molina. 2014. "Diverse
795 Responses of Forest Growth to Drought Time-Scales in the Northern Hemisphere." *Global*
796 *Ecology and Biogeography* 23 (9): 1019–30. doi:10.1111/geb.12183.

797 Vitas, A., and K. Žeimavičius. 2006. "Trends of Decline of Douglas Fir in Lithuania :
798 Dendroclimatological Approach." *Baltic Forestry* 12 (2): 200–208.

799 Walker, Lawrence R., David a. Wardle, Richard D. Bardgett, and Bruce D. Clarkson. 2010. "The
800 Use of Chronosequences in Studies of Ecological Succession and Soil Development." *Journal*
801 *of Ecology* 98 (4): 725–36. doi:10.1111/j.1365-2745.2010.01664.x.

802 Warren, Cr, Jf McGrath, and Ma Adams. 2001. "Water Availability and Carbon Isotope
803 Discrimination in Conifers." *Oecologia* 127 (4): 476–86. doi:10.1007/s004420000609.

804 Williams, David G., R. David Evans, Jason B. West, and James R. Ehleringer. 2007. *Stable*
805 *Isotopes as Indicators of Ecological Change*. *Terrestrial Ecology*. Vol. 1. doi:10.1016/S1936-
806 7961(07)01024-X.

807 Wood, Simon N. "Package ' Mgcv '." doi:10.1186/1471-2105-11-11.Bioconductor.

808 Wood, Simon N. 2017. "Package ' Mgcv .'"

809 Wood, Simon N. 2006. *Generalized Additive Models: An Introduction with R*. Chapman and
810 Hall/CRC.

811 Wood, Simon N. 2008. "Fast Stable Direct Fitting and Smoothness Selection for Generalized
812 Additive Models." *Journal of the Royal Statistical Society. Series B: Statistical Methodology*
813 70 (3): 495–518. doi:10.1111/j.1467-9868.2007.00646.x.

814 Zhao, Maosheng, and Steven W Running. 2010. "Drought-Induced Reduction in Global Terrestrial
815 Net Primary Production from 2000 Through 2009." *Science* 329 (5994): 940–43.
816 doi:10.1126/science.1192666.

817 Zweifel, Roman, Lukas Zimmermann, Fabienne Zeugin, and David M. Newbery. 2006. "Intra-

Annual Radial Growth and Water Relations of Trees: Implications towards a Growth Mechanism." *Journal of Experimental Botany* 57 (6): 1445–59. doi:10.1093/jxb/erj125.

Chapter II - Douglas fir eco-phisiological response to global change

1. Introduction

Global change has resulted in a significant alteration of forests growth over recent decades, in Europe in particular (Boisvenue and Running 2006; Zhao and Running 2010), but the main drivers and functional basis of this trend still have to be ascertained.

State-of-the-art earth system models (e.g. Sitch et al. 2008) predict a stimulation of photosynthetic rates (A_{max}) as a result of increasing atmospheric [CO_2] and temperatures, as well as atmospheric N deposition (Magnani et al. 2007), resulting in higher rates of Gross Primary Production (GPP). On the other hand, this positive effect could be negated by parallel changes in stomatal conductance (g_s) and transpiration rates, in particular in dry regions (Gómez-Guerrero et al. 2013). The balance between these two processes reflects in variations in intrinsic water-use efficiency (iWUE= A_{max}/g_s), which affects the amount of carbon gained by the tree per unit of water lost and is therefore an important functional parameter, in particular in dry climates where water acts as an important limiting factor.

At tree level, both manipulative experiments under elevated air [CO_2] (Ainsworth and Long 2005) and studies on field-growth trees, considering the effects of increasing atmospheric CO_2 in combination with a range of co-limiting factors, demonstrate an increase in iWUE as a result of global change (Peñuelas et al., 2011; Frank et al. 2015). On the other hand, contrasting results were obtained on the effects of CO_2 enrichment on growth.

As a complement to long-term experimentation the stable isotope composition recorded in wood tree rings can be considered as reliable eco-physiological proxies for retrospective analysis on

iWUE trends (Farquhar et al.,1989). Plant carbon isotopic composition ($\delta^{13}C$) is known to be directly related to iWUE and therefore affected by variations in either A or g_s, or both, while oxygen isotopic composition ($\delta^{18}O$) is mainly affected by evaporative enrichment at the leaf level, linked to g_s, but is independent from A changes. As a result, the combined analysis of both isotope signals, the so called dual isotope approach, can be used to isolate the effects of A and g_s on iWUE long-term trends (Scheidegger et al. 2000).

This would eventually make it possible to recognize if the observed trends are driven by a CO2 fertilization effect or by changes in water availability constraints.

Both growth and iWUE, however, are potentially affected also by tree age and size, possibly due to a gradual decline in g_s with the progressive increase of hydraulic resistance in taller stems (Ryan and Yoder 1997). The separation of such an (endogenous) age/size signal from the combined (exogenous) environmental signal is therefore a pre-requisite for the correct interpretation of global change impact on forest growth and iWUE.

A better understanding of the long-term reaction of forest ecosystems to the combined action of rising [CO_2] and climate covariates is determinant to forecast their future role as a carbon sink or source and the magnitude of their mitigation effect on global change. Furthermore it can provide important insights in the future behavior of economically important timber species such as Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), thus affecting management choices.

The aim of the present study is therefore

(i) to evaluate the impact of age/size and global change effects on isotopic long-term variations in Douglas fir on the Italian Appennines,

(ii) to understand the main global change components driving Douglas-fir eco-physiological response and

(iii) using the dual isotope approach to disentangle the role of changes in photosynthetic activity or stomatal conductance in recent iWUE trends.

2. Material and Methods

2.1 Study area

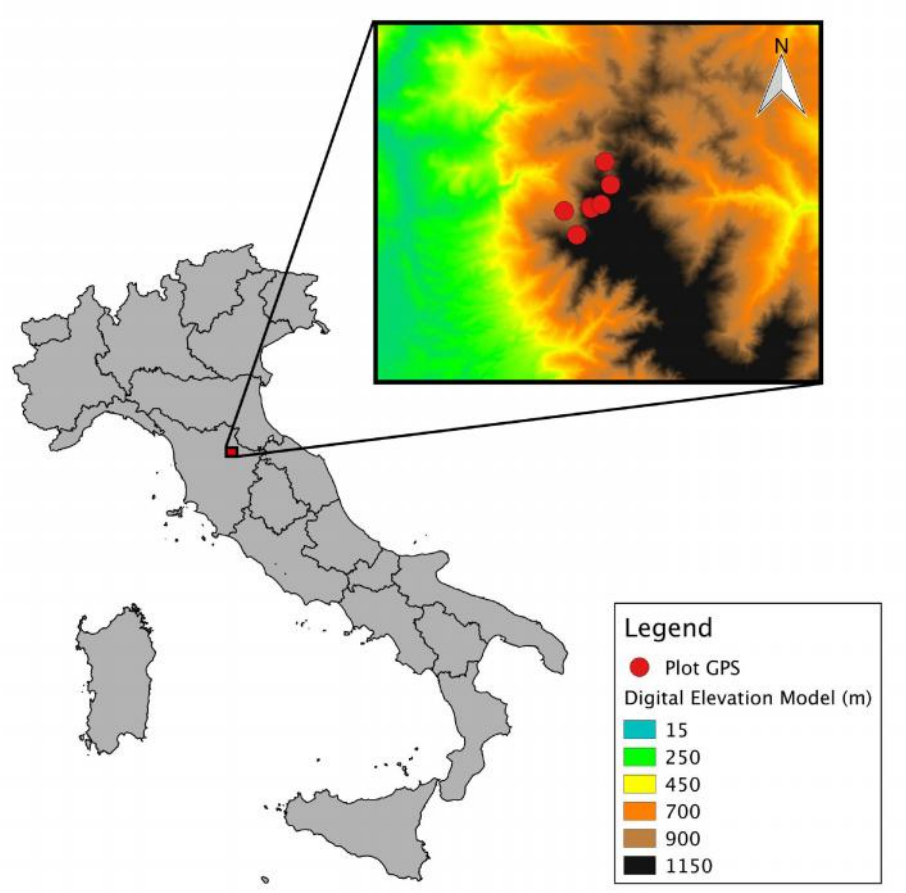


Fig.1 Map shows the location of the seven plots sampled (red dots). Different colors are related to different elevations (m, a.s.l).

This study was performed in a Douglas-fir plantation located in the Vallombrosa Forest, in the Apennine mountain range near Florence, Italy (43°43'59.6"N 11°33'16.9"E). The region has a Mediterranean climate without significant summer droughts, and the mean annual precipitation is approximately 1400 mm, of which less than 10% occurs in the summer months (72.48 mm). The mean annual temperature is 9.8°C. The soils, derived from the Macigno del Chianti sandstone series, vary between Humic Dystrudept and Typic Humudept (USDA Soil Survy Staff, 1999) in the younger and older stands, respctively, indicating similar soil conditions at the sites. Douglas-fir is a

non-indignous evergreen species and was imported from the Pacific Coast of the United States during the last decades of the 19th century. It was chosen for the present study because of its high economic importance. The sampled areas are part of the experimental permanent plot network managed by CREA Research Centre for Forestry and Wood, and include the oldest experimental plots established in Italy at the beginning of the 20th century (Pavari 1916).

A chronosequence of plots was selected for the study; a chronosequence is here defined as a set of even-aged stands growing under the same environmental conditions and differing only for their age (Walker et al. 2010). The chronosequence comprises four different age classes (with mean age 65, 80, 100 and 120 years), covering the longest temporal extension that is possible to achieve in Italy for this species. The summary characteristics of the four age classes are summarized in Table 1. Seven plots were sampled that were consistent for management, aspect and elevation. Two plots were selected for each age class, in order to ensure replication; however this was not possible for the oldest class, as only one of this age was present in the area. In all sites, only dominant trees were chosen for the analysis, in order to avoid competition effects. Data from repeated forest inventories at the sites ensured the permanence of their dominant status, thus partially avoiding potential sampling biases, which occur when the currently largest-diameter trees are wrongly considered to have always been in the dominant class (Cherubini et al. 1998). 3

Tab. 1| Mean characteristics of the Douglas fir chronosequence plots.

	Age-class						
	120	100			80	65	
Plot (code)	1	2	3	4	5	6	7
Max age (yrs)	126	101	102	86	86	69	69
Elevation (a.s.l.)	900	1100	1009	1095	1280	1113	1113
Esposition	N	N	SW	NE	NE	SW	SW
Dominant diameter (cm)	--	60.8	67.5	58.2	61,5	50.3	43.3
Dominant high (m)	--	47.2	54.4	49.9	44.3	39.9	39.8
Stand density (n°ha-1)	30*	375	380	360	280	600	550
Trees sampled (n°)	5	5	5	5	5	5	5

*for the oldest plot only 30 plants are left standing

2.2 Samples preparation and isotopic analysis

Stable isotopes series were measured for individual trees at 5-years resolution. After cross-dating (following the procedure described in chapter I), cores were subdivided in 5-rings blocks with a scalpel under a stereo-microscope. The blocks were subsequently ground with a ball-mill (MM400, Retsch, Germany) and pooled together. No fixative (glue or adhesive tape) or substance to highlight the rings (dye or chalk) were used during tree-rings analysis, so as to avoid any potential chemical contamination (Williams et al. 2007). All analyses were performed on whole wood rather than α-cellulose samples; however, since lignin or other mobile compounds (i.e. resins, oils and hemi-cellulose) could be deposited after the year of ring formation, so leading to biological misinterpretation, the isotopic composition of both whole wood and α-cellulose was measured on a representative sub-set of samples (n=35, coming from 7 different trees, one for each plot). The calibration equation derived from the comparison of the two groups was used to correct all measurements for a constant offset as discussed in Warren (2001; Fig.1 and 2). The α-cellulose extraction method adopted was the one proposed by Boettger (2007); 10 mg of fine-powdered wood samples were weighted and sealed in Teflon bags. In a first step, samples were incubated twice for 2 hours at 60 °C in a 5% NaOH solution to remove fats, oils, resins, tannins and some hemi-cellulose. Afterward, samples were washed three times with boiling deionizer water to stop the reaction. In a second step, lignin was removed with a 7 % $NaClO_2$solution, with the addition of 8-16 ml of acetic acid (CH_3OOH) per liter, until the solution achieved a pH level between 4-5. The reaction was run for 36 h at 60°C, changing the solution every 10 hours. Finally, samples were washed three times in boiling deionizer water and dried at 50 °C. For ach sample, an amount of 0.250 mg ±0.02 and 0.55 ±0.03 was weighted into tin or silver cups for carbon (C) or oxygen (O) analysis, respectively. A total of 1222 (n=611 for both C and O) whole-wood samples were measured, in addition to the 70 tested for pairwise comparison of α-cellulose against whole wood measurements. Carbon stable isotope analysis were performed in continuous flow-isotope ratio mass spectrometry (CF-IRMS), with an isotopic mass spectrometer (DELTA Plus, Thermofisher)

interfaced to an elemental analyzer. Oxygen stable isotopes were measured in an elemental analyzer (EA-1108, Carlo Erba Thermoquest, Milan, Italy), after decomposition into CO of the material with thermal pyrolysis at 1080°C and analyzed with a isotope ratio mass spectrometer (Delta Plus XP, Thermo Finnigan) also in continuous flow. The precision of the analyses was ±0.1‰ for carbon and ±0.2‰ for oxygen, and raw data were expressed as relative deviation from the international standards V-PDB (Vienna Pee Dee Belemnite for $^{13}C/^{12}C$) and V-SMOW (Vienna Standard Mean Ocean Water for $^{18}O/^{16}O$).

2.3 $\delta^{13}C$ theory

Carbon isotopic composition ($\delta^{13}C$) in tree rings provides an integrative measurement of the whole tree photosynthetic and gas exchange activity throughout the period the wood was synthesized (Todd E. Dawson et al. 2002). In fact, plants tend to discriminate against $^{13}CO_2$, therefore the resulting organic matter is enriched in the lighter isotope, ^{12}C, compared to atmosphere. The level of this enrichment is mediated by environmental conditions, such as temperature and water availability, which influence the conductance (g_s), but also by biogeochemical factors affecting photosynthesis (A_{max}), such as atmospheric CO_2 concentration or N deposition. Thus, the analysis of carbon isotope discrimination recorded in tree rings can provide a dated sourc of information useful to reconstruct tree long-term physiological activity. Sample isotopic composition is expressed as the relative abundance $^{13}C/^{12}C$ of the sample (R_{sample}) compared to the V-PDB standard ($R_{standard}$)

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1]‰ \quad (1)$$

To remove the effect of the atmospheric decline in $\delta^{13}C$ caused by anthropogenic emissions (typically depleted in $^{13}CO_2$), the so called “Suess effect” (Francey et al. 1999), the $\delta^{13}C$ composition was expressed in terms of discrimination against ^{13}C by the equation:

$$\Delta^{13}C = (\delta^{13}C_a - \delta^{13}C_p)/(1+ \delta^{13}C_p) \quad (2)$$

where $\delta^{13}C_a$ and $\delta^{13}C_p$ are the isotopic composition of carbon of atmospheric CO_2 and plant material, respectively. The stable isotope discrimination of plants tissue was related to c_i/c_a, trough the linear model developed by Farquhar (1989) as:

$$\Delta^{13}C = a + (b-a)\, c_i/c_a \quad (3)$$

where a is the isotopic fractionation during photosynthetic gas exchange caused by the slower diffusion of $^{13}CO_2$ through stomata (4.4‰), b is the net fractionation associated with RuBP carboxylase activity (27‰), and c_a and c_i are the atmospheric and intercellular [CO_2]. On the other hand, the c_i/c_a ratio can also be linked to changes in intrinsic water-use efficiency (iWUE), defined as the ratio between maximum photosynthetic capacity (A_{max}) and stomatal conductance (g_s):

$$iWUE = A_{max} / g_s = (c_a - c_i) / 1.6 \quad (4)$$

As a result, it is possible to infer iWUE changes from $\Delta^{13}C$ as:

$$iWUE = 0.625\, c_a[\, 1 - (\, \Delta^{13}C - a\,) / (\, b - a\,)\,] \quad (5)$$

Mean annual $\delta^{13}C_a$ values used in the present study were obtained from the NOAA Earth System Research Laboratory, recorded at the Mauna Loa observatory in Hawaii from 1959 to present, integrated with those published by McCarroll and Loader (2004) for previous years.

2.4 $\delta^{18}O$ theory

Tree-ring oxygen isotope composition depends on the one hand on the $\delta^{18}O$ signal of the water source used by the tree, on the other hand on evaporative enrichment, which occurs at leaf level as a result of transpiration (Sternberg 2009). The primary source of water for forest trees is typically meteoric, but its $\delta^{18}O$ signature can vary in space and time. Temporal variations at an inter-annual scale are related to climatic factors; for example warmer years are characterized by an enrichment in the composition of meteoric water due to higher proportion of $H_2{}^{18}O$ molecules evaporated from oceans (Rozanski et al,.1992). Morover, intra-annual variations in plants' main source of water (precipitation, ground water, fog or snow), as well as seasonal isotopic changes of these sources themselves, due to temperature dependent fractionation, can determine an additional shift in the input signal (Todd E. Dawson and Pate 1996). The spatial variation in precipitation $\delta^{18}O$ is influenced by both distance from the sea and elevation (Giustini et al., 2016): as air moves from a lower to a higher region, the temperature differential produces an isotope vertical gradient. This altitude-dependent fractionation is determined by the progressive depletion from the rain of the $H_2{}^{18}O$, which tends to condensate faster relative to to $H_2{}^{16}O$. Once in the soil, water tends to become gradually enriched in the heavier isotope ($H_2{}^{18}O$) close to the surface (0.1-0.5 m). This process is caused by the preferential depletion in $H_2{}^{16}O$ due to evaporative effect and it is enhanced by the residence time of water in the ground. Deeper moisture remains unaffected, mirroring the system water source composition (Brunel et al. 1991). As a result, the depth at which root systems are tapping water can determine the input isotopic signal and it could change over the life of the tree as the root system grows deeper. This is one of the possible causes proposed to explain a possible age-related $\delta^{18}O$ decreasing trend observed in trees (Esper et al. 2010). No additional isotopic fractionation is expected when water enters into roots and during its transport trough the xylem to the leaves (Dawson and Ehleringer, 1991).

Secondly, a process of evaporative enrichment relative to the source of water takes place at the leaf level (Dongmann and Nürnberg 1974). The mechanistic model proposed by Craig and Gordon

(1965) to explain the degree of enrichment at the evaporation sites ($\Delta^{18}O_e$) above the xylem water predicts that:

$$\Delta^{18}O_e = \varepsilon^* + \varepsilon_k + (\Delta^{18}O_v - \varepsilon_k) * e_a/e_i \quad (6)$$

where ε^* is the (temperature dependent) equilibrium fractionation between liquid water and water vapour, ε_k is the kinetic fractionation during water diffusion through stomata and the leaf boundary layer, $\Delta^{18}O_v$ is the water vapour isotope composition in the air relative to the source of water (= $\delta^{18}O_v - \delta^{18}O_s$) and e_a and e_i are the ambient and intercellular vapour pressure, respectively. This equation highlights the inverse relationship which exists between air relative humidity (RH), which influences e_a/e_i, and $\delta^{18}O$ in leaf water. The degree of leaf water enrichment is expected to be directly related to transpiration (E) rates, if this variation is mainly driven by evaporative demand (Farquhar et al., 2006). However, this model was found to overestimate the degree of enrichment due to the failure to consider the mixing of $\delta^{18}O$ of enriched water at evaporation sites with un-enriched water from the xylem, known as Péclet effect (Farquhar and Lloyd 1993). This tends to be higher at high transpiration rates (greater xylematic flow), so decreasing the evaporative enrichment at leaf level. In this way the Péclet effect increases the importance of $\delta^{18}O_s$ in determining the $\delta^{18}O_{cel}$, as described by Barbour and Farquhar (2000):

$$\delta^{18}O_{cel} = \delta^{18}O_s (P_{ex} * P_x) + \delta^{18}O_l (1 - P_{ex} * P_x) + \varepsilon_{wc} \quad (7)$$

where ε_{wc} is the equilibrium fractionation between water and carbonyl groups , P_{ex} is the proportion of exchangeable oxygen in cellulose and P_x is the proportion of xylem water in the meristematic tissue where cellulose is synthesized, whereas $\delta^{18}O_l$ is the leaf water isotopic composition. This

1034 equation highlights the inverse relationship between $\delta^{18}O_{cel}$ and stomatal conductance (Grams et al.
1035 2007).

1036 Assuming that the relative strength of evaporative enrichment at the leaf level is preserved and
1037 overshadows the source $\delta^{18}O$ signal (meteoric), this information could be used to infer the response
1038 of tree stomatal conductance to environmental factors.

1039

1040 ## 2.5 Dual isotope conceptual model

1041 The limit in the application of iWUE as a proxy of tree eco-physiological response to environmental
1042 or biochemical forcing is that its variations cannot be attributed unambiguously to changes in either
1043 A_{max} or g_s. For example, a reduction in the $\delta^{13}C$ values observed in organic matter can be ascribed
1044 to a rise in c_i level and therefore to a reduction in iWUE. This reduction can be ascribed either (i) to
1045 a decrease in photosynthetic activity (at constant g_s) or (ii) to an enhancement in stomatal
1046 conductance (at constant A_{max}). This limitation can be overcome through the application of the
1047 qualitative model proposed by Scheidegger et al. (2000), so as to try to disentangle which factor is
1048 driving the observed iWUE trend. The model enables the deduction of changes in g_s and A_{max} in
1049 subsequent time intervals from different C and O isotope composition patterns. The eight possible
1050 combinations are depicted in the central part of the scheme presented in Fig. 1, representing the
1051 observed patterns, while the arrows in the external boxeshighlight the most likely interpretation of
1052 these scenarios. Due to the temporal changes in atmospheric $\delta^{13}C_a$ (Suess effect) a correction was
1053 preliminarily performed by adding to each $\delta^{13}C_p$ value a factor corresponding to the deviation of the
1054 corresponding annual $\delta^{13}C_a$ value from the pre-industrial reference value of -6.4% (McCarroll et al.,
1055 2004). Furthermore, each isotopic series was normalized in respect to its mean to remove infra-
1056 series variability and the possible effect of different water sources among plots for $\delta^{18}O$ (Barnard et
1057 al.2012).

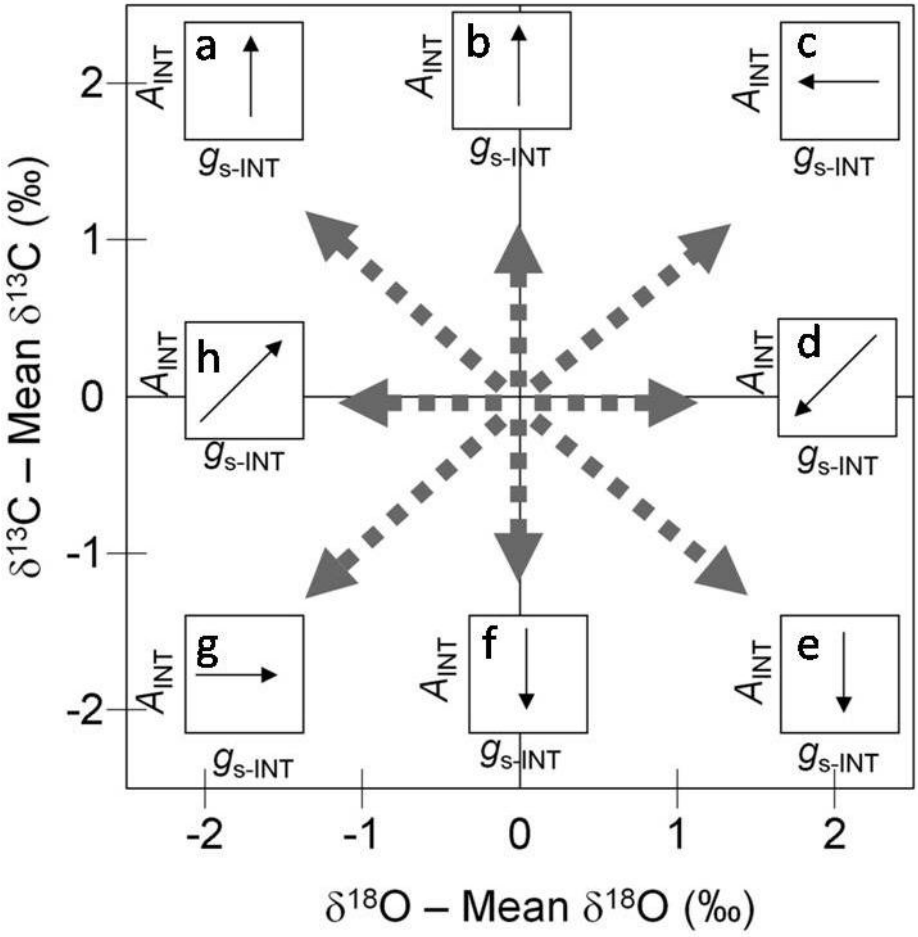


Fig.1 Dual isotope conceptual model scheme. Scheidegger model for relationship interpretation between $\delta^{13}C$ and $\delta^{18}O$ (here expressed as deviation from the mean). The central part of the pictures summarize the 8 possible directions in which temporal variations can move (between time periods). Each arrow points on the "most likely case", explaining the behavior of A_{-INT} (A_{max} integrated on the considered period) and $g_{s\ -INT}$ (g_s integrated on the considered period). From Barnard et al 2012

2.6 GAM model

Since iWUE can potentially show non-linear patterns in response to both biological (i.e. age/size and juvenile effect) and environmental (i.e. temperature and precipitation, geochemical variables) drivers, Genralized Additive Models (GAMs; (Hastie and Tibshirani 1990) were applied to conveniently capture the shape of the inherent relationships existing between iWUE and predictor variables, and the superposition of their effects. GAMs are non-linear regression models that

specify the value of the dependent variables as the sum of smooth functions of a numbr of independent variables in a non-parametric fashion. Such a model relaxates the *a priori* assumption of a linear functional relationship between response and predictors that is central to multiple linear regression models, therefore resulting in a more flexible range of the application. Th relationship is expressed as:

$$y_i = X_i \beta + s_1(x_{i1}) + \cdots + s_n(x_{in}) + \varepsilon_i \quad \text{where } \varepsilon i \sim N(0,\sigma^2) \tag{8}$$

where y_i is the i-th value of the response variable, β is a vector of fixed parameters**,** Xi is the fixed i rows of the model matrix**,** $x_1,\ldots,x_n$ are independent variables, $s_1,\ldots,sn$ are smooth functions of the independent variables and εi are residuals with Gaussian normal distribution and constant variance. A cubic penalized spline was used as a smooth function. This is the result of the simultaneous fitting of basis functions (i.e. natural cubic spline) penalized to achieve the optimal degree of smoothness, avoiding data over-fitting. The amount of penalization was automatically computed by the maximum likelihood estimation (ML; Wood and Wood 2013). The covariate selection was performed by a stepwise backward process. Tree age, atmospheric [CO_2], total atmospheric N deposition or its NH_x and NO_y components, mean (T_m) or maximum (T_{max}) and minimum (T_{min}) annual temperatures, annual precipitation (P) and the SPEI value of the current and previous year ($SPEI_{t-1}$) were considered as possible covariates. Candidates for removal were identified through their lower approximate p-values, and the model resulting from the subtraction of such variable was compared with the previous one in terms of their Bayesan information criterion (BIC). This index was used instead of the Akaike's information criterion (AIC) because it is less conservative and more useful to assess the 'true' model in confirmatory analysis; indeed in model selection, the BIC provides a better opportunity to understand which pool of variables generated the real data (Aho, Derryberry, and Peterson 2014). The GAMs analysis was performed with the mgcv pakage (Wood

2006) using the R statistical suite (R Core Team, 2017). No pre-whitening processes (i.e addition of an AR1 model for the correction of residuals autocorrelation) were applied to the iWUE and $\delta^{18}O$ time series, with the aim to preserve the true long-term trend. The concurvity level (i.e. the generalization of non-linear models' co-linearity) was also checked to assess a potential correlation among independent variables. It could be an issue in models including a time-dependent smooth function with other time-varying covariates, making model estimation unstable (Wood, 2006). Nevertheless, GAMs are able to deal with some degree of concurvity (Wood, 2008). Finally, the model was validated to ensure that the assumption of normal distribution of observations and the absence of heteroscedasticity of residuals were respected.

3. Results

3.1 Effects of cellulose extraction

Our preliminary comparison of C and O isotope composition on paired whole-wood or -cellulose samples (Fig. 2) confirms the possibility of obtaining unbiased results from untreated material, when interested in temporal dynamics rather than absolute values. A very tight relationship between extracted and unextracted samples was observed in the case of $\delta^{13}C$ (Fig. 2a; $R^2 = 0.96$), although with a slope significantly different from unity. The relationship could be therefore used to estimate the cellulose isotopic value from whole-wood measurements, using this value for the estimation of iWUE. A worse relationship was observed in the case of $\delta^{118}O$ (Fig. 1b; $R^2 = 0.65$), although a consistent relationship was observed within each wood core, demonstrating the possibility to reconstruct the pattern of long-term tree dynamics from unextracted samples.

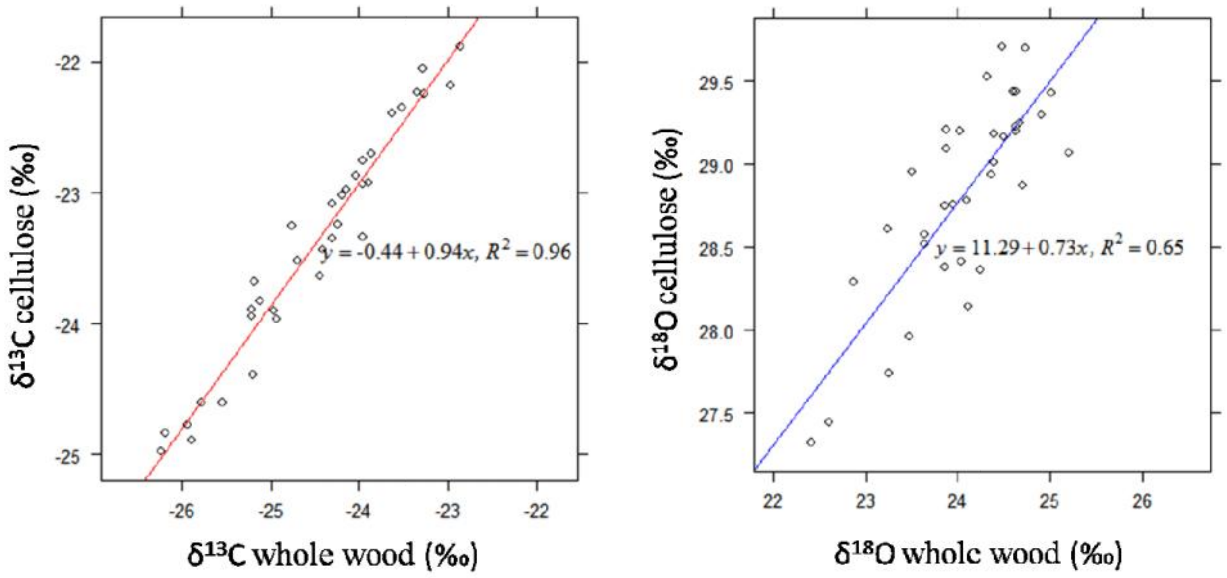


Fig.2 a, b isotope composition on extracted against un-extracted. Relationship between $\delta^{13}C$ (red) and $\delta^{18}O$ (blue) in cellulose (‰),on *y*-axis, and in whole wood (‰),on *x*-axis, of the test's sub-set (n=35). The equations were used to correct the offsets in whole wood values on the main data-sets.

3.2 Carbon isotope and iWUE dynamics

Trees were grouped into four age classes, based on their age at the time of sampling (approximately 65, 85, 100 and 120 years), The attributes (iWUE, $\delta^{13}C$, $\delta^{18}O$) and relative statistics of each Douglas-fir cohort are summarized in Table 1. Across the entire dataset, estimated iWUE ranged between 70.09 and 138.44, as a result of inter-individual differenc es as well as time dynamics; the associated range in $\delta^{13}C$ was between -21.87 and -26.58, while $\delta^{18}O$ varied between 22.10 and 26.44 in the oldest age class.

The iWUE raw time series (Fig. 3) show rather coherent trends between all the different age-classes, confirming the reliability of the measurements. When considering the time pattern of the variable (values aligned by calendar year), iWUE shows an increasing trend during the period from 1960 to 1980 for all age-classes (coloured lines, representing a spline curve fitted on all the data of

each cohort), followed by a less pronounced growth in recent decades (Fig.3). Higher iWUE values were achieved by the older class, even if only in a slightly significant way, but no clear separation among age-classes is apparent, making it difficult to attribute the pattern to either age or variable environmntal conditions over time.

Taking a diachronic view (values aligned by cambial age), the younger classes exhibit remarkably higher valuesat any given age, in comparison with the older ones (Fig.4), underling a possible effect due to changing environmental condition over time.

Tab.1 |Descriptive statistics for water use efficiency (iWUE), oxygen composition ($\delta^{18}O$) and carbon composition ($\delta^{13}C$) chronologies of the four age-classes in which trees were grouped.

Var	Age-class	*N*	*Mean*	*SD*	*Median*	*Min*	*Max*
iWUE	65	63	107.44	11.27	107.89	89.34	128.77
	85	172	100.95	11.23	101.19	76.82	124.22
	100	210	102.10	12.76	100.52	70.09	137.31
	120	166	101.37	14.52	99.69	76.76	138.44
$\delta^{18}O$	65	63	24.24	0.70	24.29	22.42	25.66
	85	172	23.92	0.80	23.98	22.10	25.60
	100	210	24.33	0.56	24.27	22.92	25.78
	120	166	24.99	0.80	25.15	22.98	26.44
$\delta^{13}C$	65	63	-23.89	0.66	-23.95	-25.43	-22.72
	85	172	-24.24	0.72	-24.21	-25.97	-22.41
	100	210	-23.93	0.85	-23.89	-26.58	-22.09
	120	166	-23.74	0.78	-23.00	-24.97	-21.87

n is the number of 5-yr ring blocks for each age-class, *Mean* is the mean value, *SD* is standard deviation, *Median* is the median value, *Min* is minimum value, *Max* is the maximum value, *Var* is the variable considered.

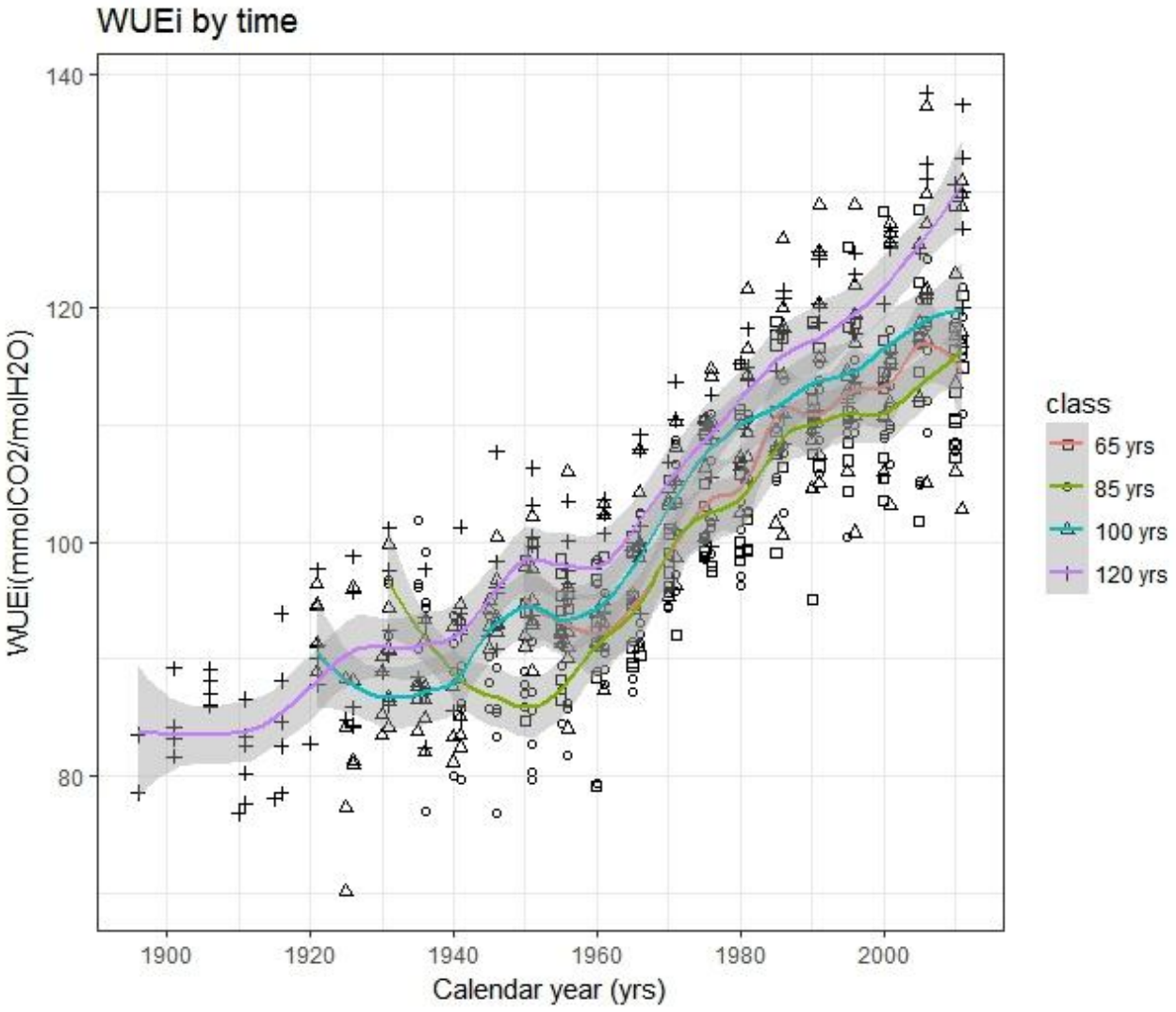


Fig.3 Time-related dynamics of iWUE in different age-classes. Lines represent splines fitted to differnt age classes. The shaded areas indicate the 95% prediction interval of the spline function

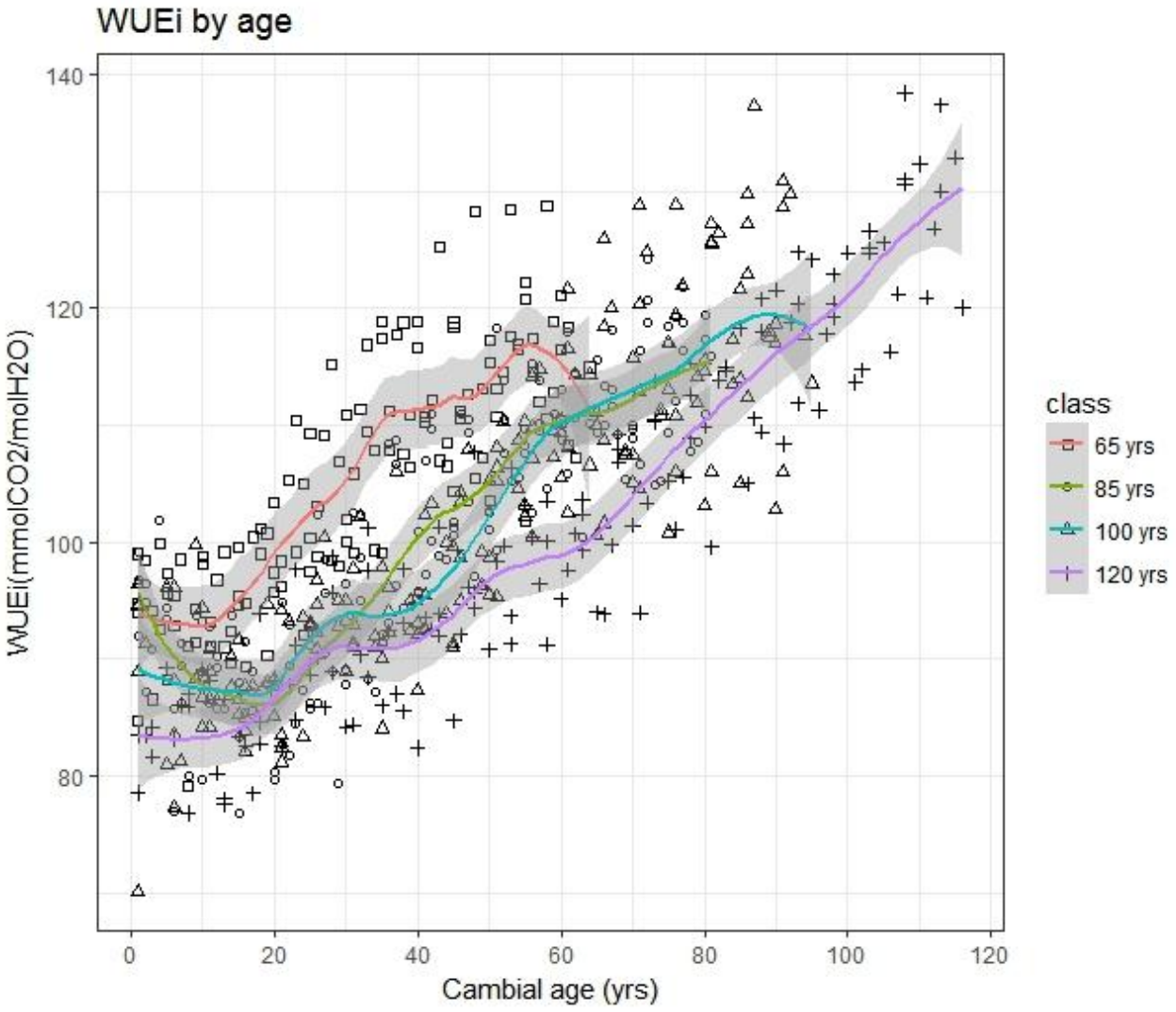


Fig.4 Diachronic analysis of age effects on iWUE in different age-classes. Lines represent splines fitted to differnt age classes. The shaded areas indicate the 95% prediction interval of the spline function..

Subsequently, in order to quantify the strength of this possible environmntal forcing, a GAM model was applied to the data. In a first step, to assess the possible change of water use efficiency over time, decoupled from the influence of age, iWUE was modeled as:

$$iWUE = s(Age) + s(TIME) + \varepsilon_i \quad (9)$$

where s(AGE) is the cambial age effect and s(TIME) represents all of the environmental and geochemical effects cumulated into a single global variable, varying along time (as in chapter I).

The variation of iWUE as a function of time alone, i.e. removing the co-occurring effects of age, shows an increase of the 17.3 % in the period included between 1960-1980, with a near stabilization afterwards (Fig. 5b). On the other hand the age-smoother displays a brief initial decreasing trend, followed by a quasi-constant increase with increasing age (Fig. 5a).

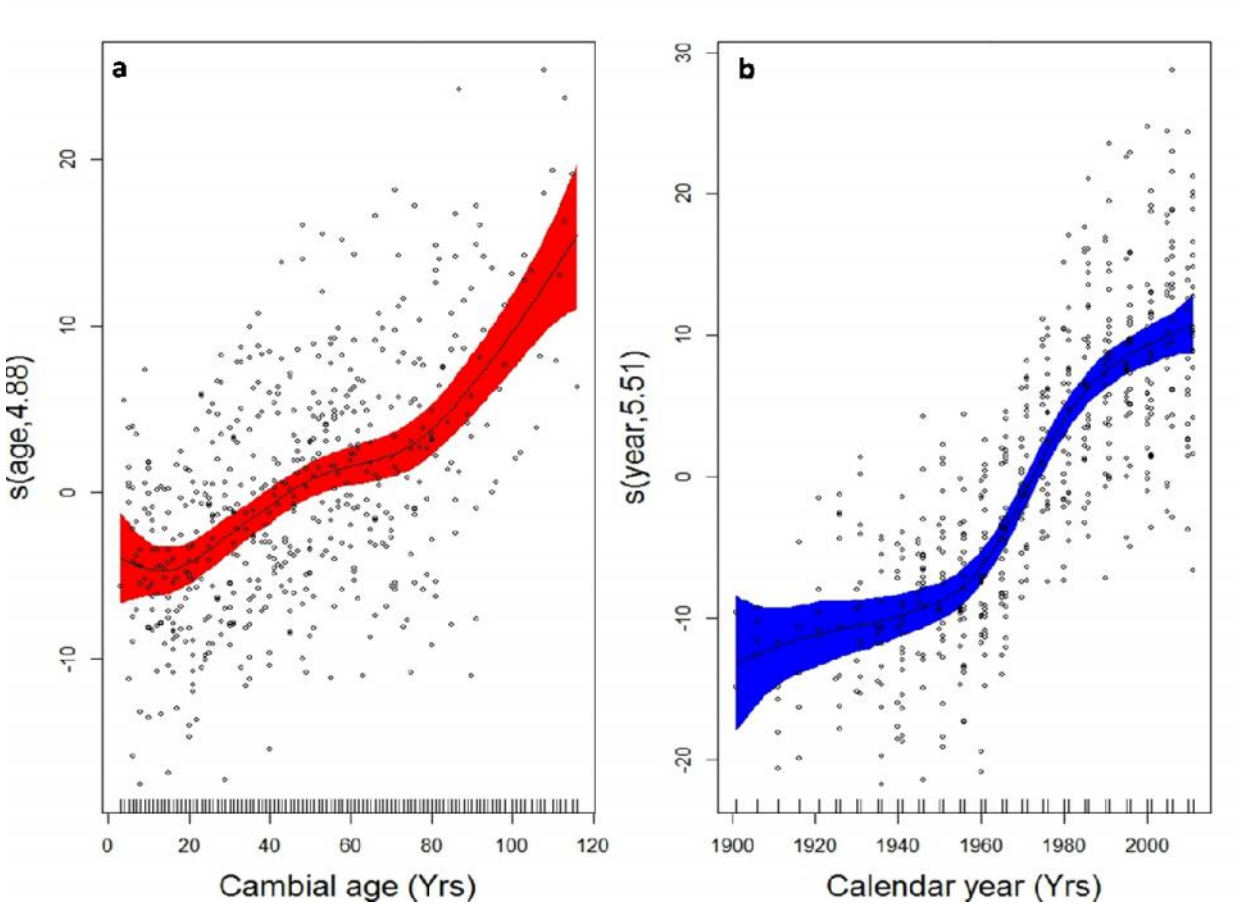


Fig.5 GAM analysis of the independent effects on iWUE of age and time .

a. Global trend of iWUE in time decupled by age-related effects. On *x*-axis time (Yrs),and on *y*-axis the function of time *s*(TIME), dimensionless and centered around 0. The shaded areas indicate the 95% confidence interval.

b. Trend of iWUE along age decupled by time-related effects. On *x*-axis age (Yrs), and on *y*-axis the function of age *f*(AGE), dimensionless and centered around 0. The shaded areas indicate the 95% confidence interval

As a final step, in order to attribute the global change effect to changes in individual environmental variables, a number of candidate annual climatic and geochemical variables were added to the

model instead of the time variable; after a backward stepwise selection, the selected model could be eventually specified as follows (Fig. 6):

$$iWUE = s(CO_2) + s(Age) + s(SPEI\ JJA\ t\text{-}1) + s(NO_{y\ dep}) + \varepsilon_i \quad (10)$$

where CO_2 is the annual level of atmospheric [CO_2], Age is the age/size effect associated with variations in cambial age, "SPEI JJA t-1" represents the August SPEI (Standardized Precipitation-Evapotranspiration Index; Vicente-Serrano et al. 2010) values in the previous summer, cumulated over the preceding 3-months period and $NO_{y\ dep}$ is the annual sum of dry and wet deposition of oxide N (NO_y) species. All variables exhibit a significant p-value at 0.001 level, with the exception of the SPEI factor, which is significant at the 0.005 level (Tab. 2). The global adjusted R^2 for the whole model is 0.795. The most relevant biological factor that explains the long-term trend in the Douglas-fir iWUE is the Age covariate (F-test= 12.970), followed by the CO_2 atmospheric concentration, the nitrogen deposition and finally by the drought index of the previous summer. Model goodness-of-fit and respect of the model assumptions were also evaluated (Fig.7).

Tab. 2 Generalized additive model results. Environmental and biological covariates' relationships with iWUE series (as dependent variable) in *Pseudotsuga menziesii.*

Factor	*e.d.f.*	*F*	*P*	$R^2(adj)$
CO_2	1.672	4.628	7.28E-12	
Age	5.395	12.97	< 2e-16	
SPEI JJA t-1	1.278	0.998	0.00127	
$NO_{y\ dep}$	2.34	3.411	8.71E-13	
				0.795

e.d.f. are effective degree of freedom, *F* is the F-test for variance explained, *P* is the p-values and $R^2(adj)$ is the adjusted regression coefficient of the model.

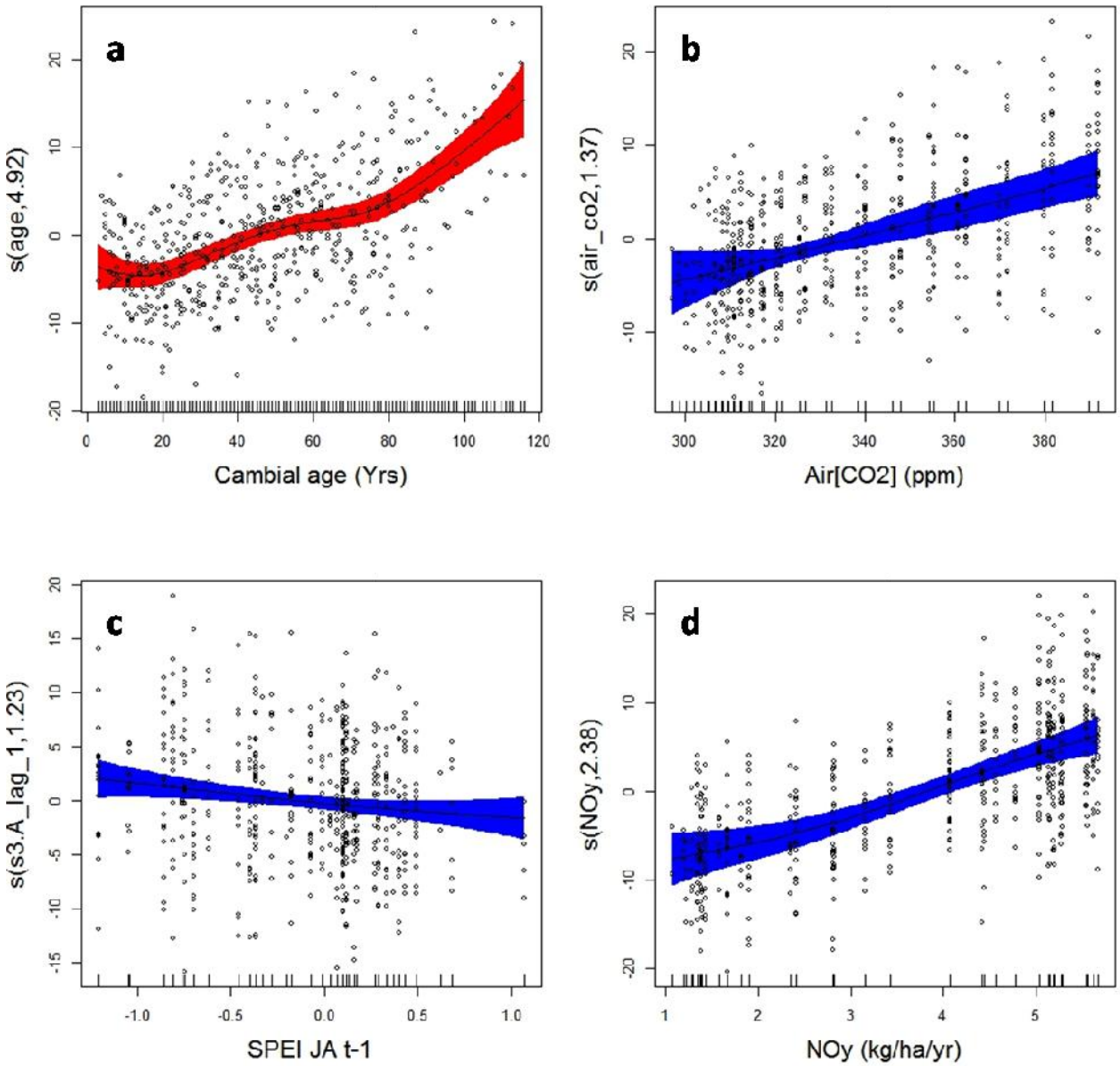


Fig. 6. GAM's results for iWUE. Generalized additive models (GAMs) results show the relationship between iWUE and environmental and biologic factors. On *y*-axis values indicate the x covariate effect on the tree rings- iWUE predicted by the model (continuous line) dimensionless and centered around 0, plus the estimated degree of freedom (edf). On *x*-axis each x variable values. Points represent partial residuals from the fitted function and the shaded areas indicate the 95% confidence interval.

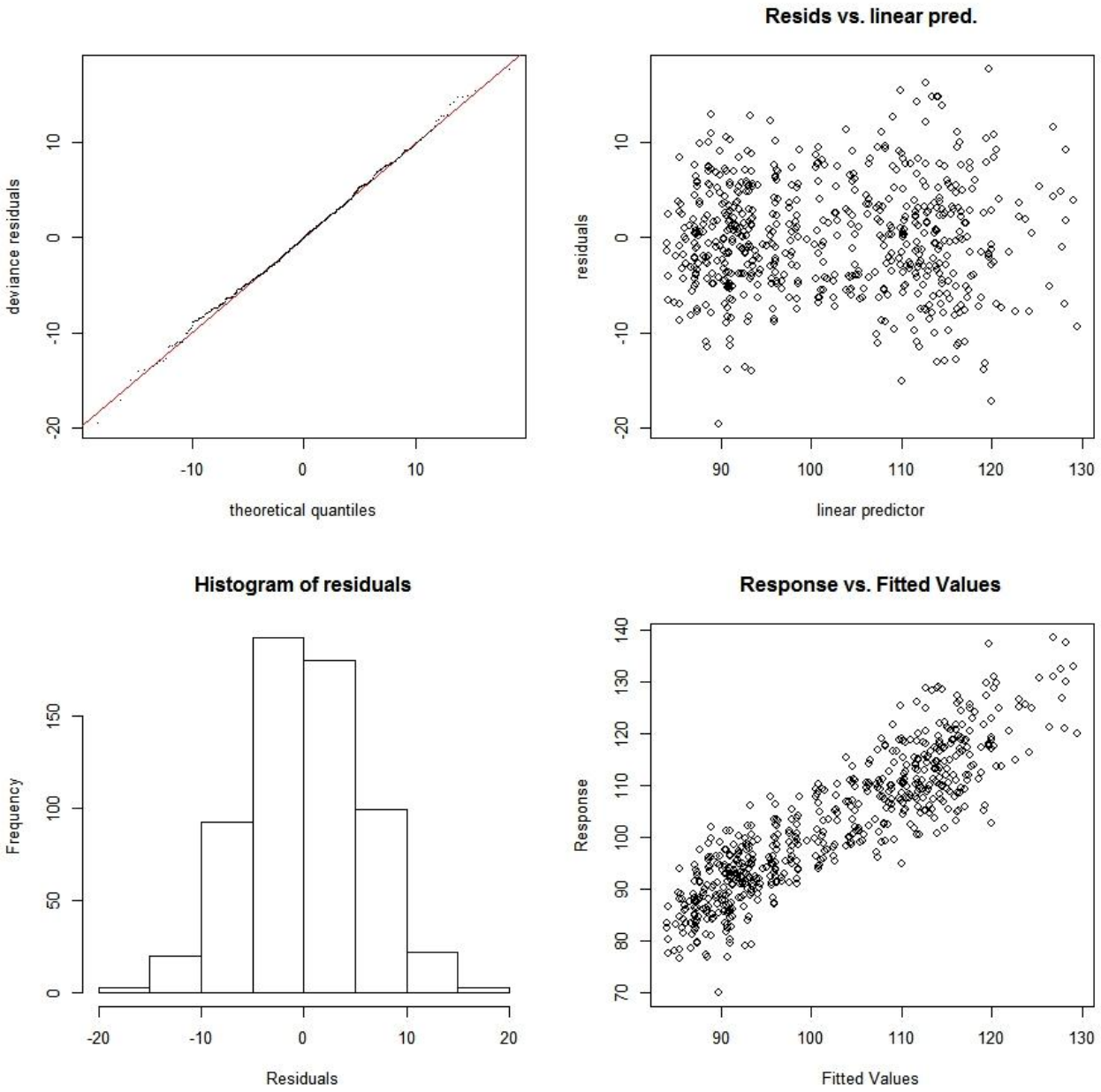


Fig.7 iWUE's GAMs model assessment. Residual distribution of the whole model selected by a stepwise procedure and against linear predictor. Response against fitted values for the whole model

3.3 Oxygen isotope dynamics

In contrast with C isotopes, $\delta^{18}O$ values for the different age-classes show a pronounced shift among the old class and the others but no clear trends over time on raw series (Fig.8). However, all the classes seem to be roughly aligned with two major peaks, corresponding to the periods when the two biggest drought events of the last century have occurred (year 1945 and in particular 2003).

Considering the apparent pattern against age (Fig. 9), no clear trend is apparent, apart from a general increase at young age.

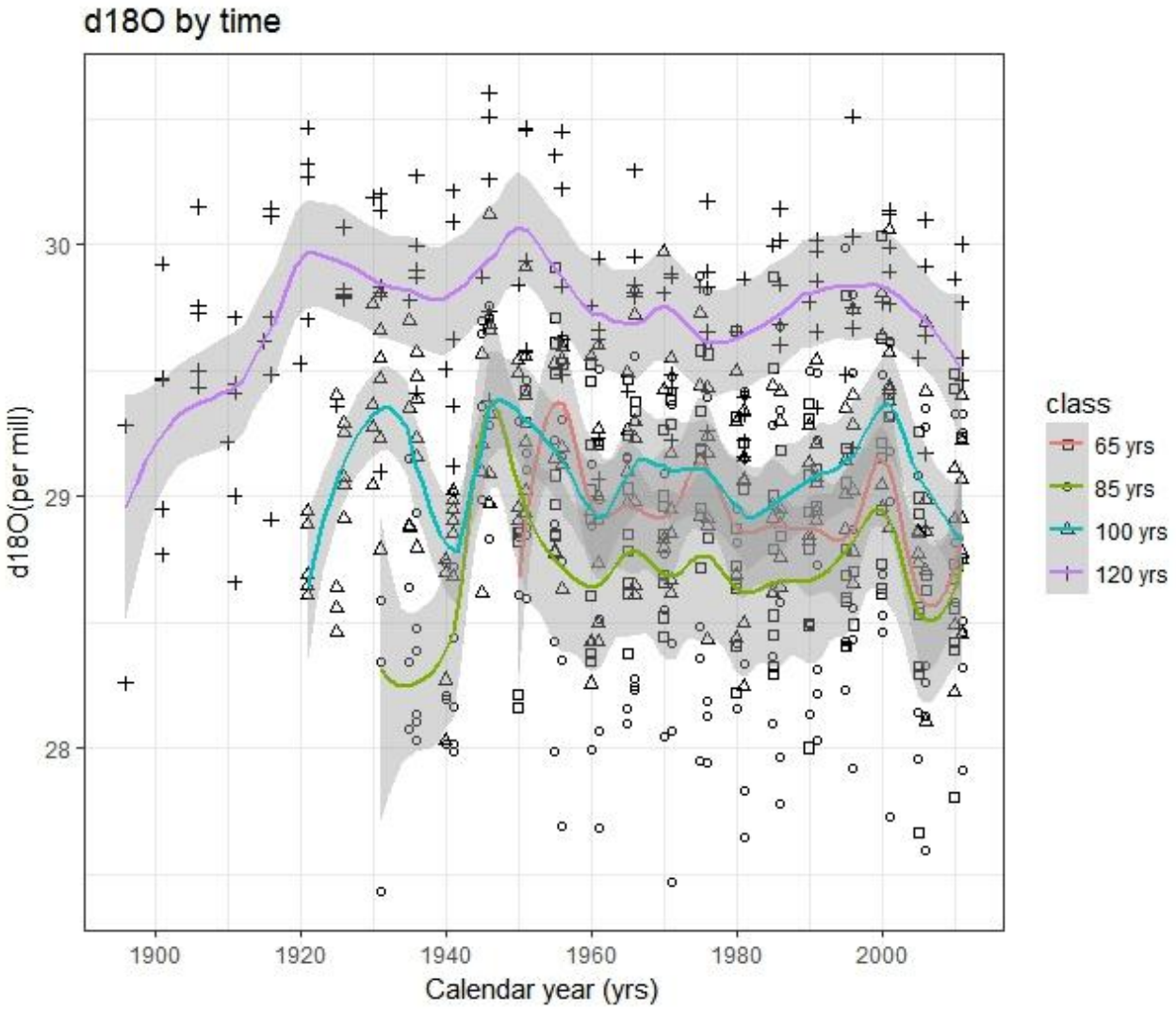


Fig.8 Time-relatd dynamics of δ^{18}Oin different age-classes. $\delta^{18}O$ time series, grouped by age-classes and fitted with a cubic spline. The shaded areas indicate the 95% prediction interval of the function

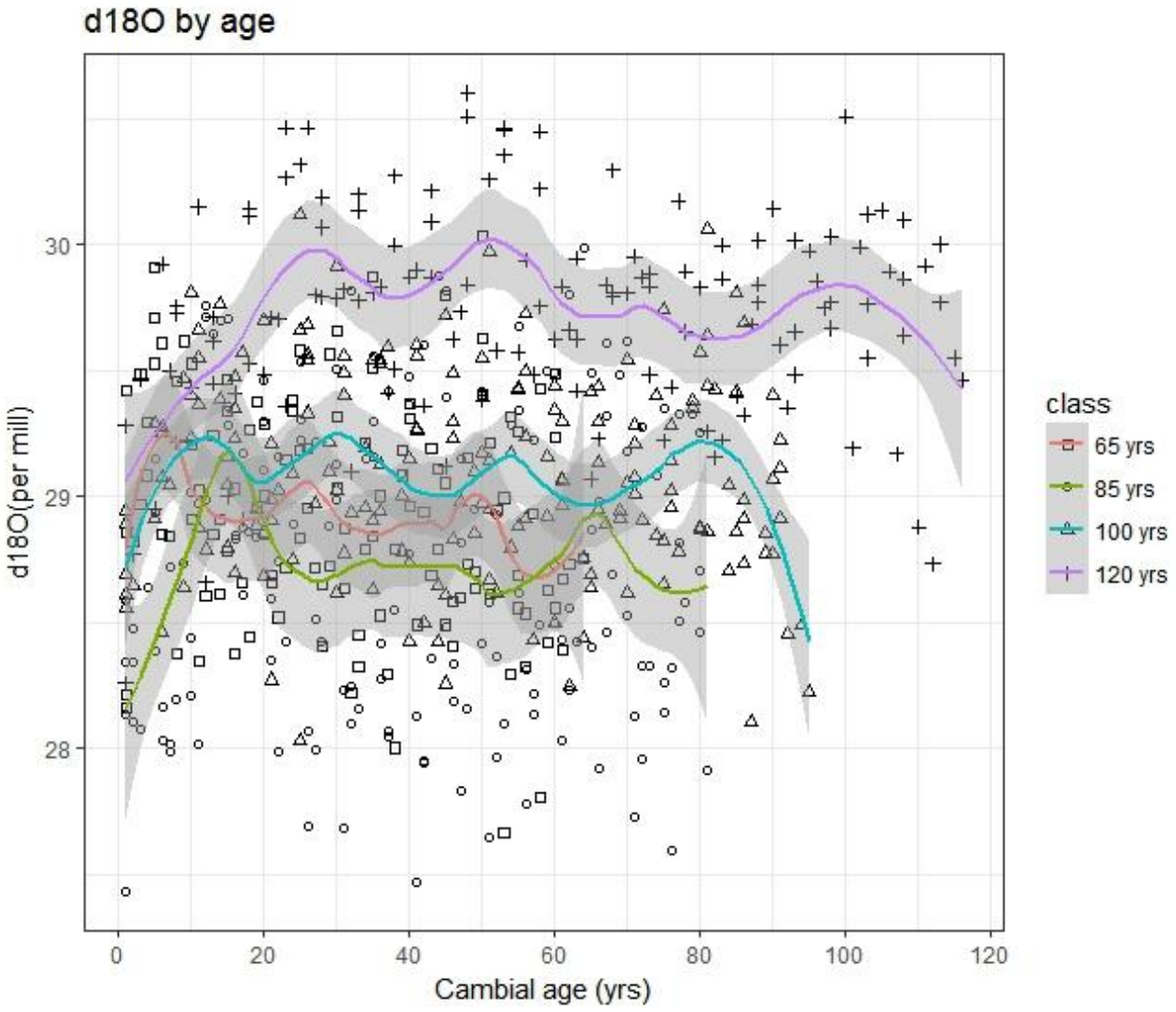


Fig.9 Diachronic analysis of age effects on $\delta^{18}O$ in different age-classes. $\delta^{18}O$ time series, grouped by age-classes and fitted with a cubic spline. $\delta^{18}O$ (‰),on *y*-axis, and cambial age (Yrs), on *x*-axis. The shaded areas indicate the 95% prediction interval of the spline function

This is confirmed by the preliminary GAMs analysis, which highlighted a significant effect of time but not of age *per se*. When applying GAMs to explain directly the time-dependent behavior of O isotopic composition in terms of individual global change drivers, the model specification after the backward variable selection procedure was:

$$\delta^{18}O = s(CO2) + s(T_{max}) + s(P_{sum}) + \text{factor(elevation)} + \varepsilon_i \quad (11)$$

where CO_2 is the annual level of atmospheric [CO_2], T_{max} is the mean of annual maximum temperature over the 5-yr period, P_{sum} is the sum of annual precipitation, and elevation is a

parametric term which considers each plot's elevation above sea level as a factor (Fig. 10). Only CO_2, among all variables, is not significant at the 0.001 p-level, but only at the 0.05 level. It's worth noting that the effect of elevation (Fig. 10d) appears to account to a large extent for the higher values observed in the oldest age class in Fig. 8 and 9. The adjusted R^2 for the whole model is 0.458 and the most relevant variable which explains the $\delta^{18}O$ patterns appears to be the annual sum of precipitation (F-test = 2.036). A test of model assumptions demonstrated a general lack of bias (Fig.11).

Tab. 3 Generalized additive model results. Environmental and biological covariates' relationships with $\delta^{18}O$ series (as dependent variable) in *Pseudotsuga menziesii.*

Factor	*e.d.f.*	*F*	*P*	$R^2(adj)$
CO_2	1.534	0.997	0.001334	
T_{max}	1.972	1.243	0.000664	
P_{sum}	3.613	2.036	0.000137	
				0.458

e.d.f. are effective degree of freedom, *F* is the F-test for variance explained, *P* is the p-values and $R^2(adj)$ is the adjusted regression coefficient of the model.

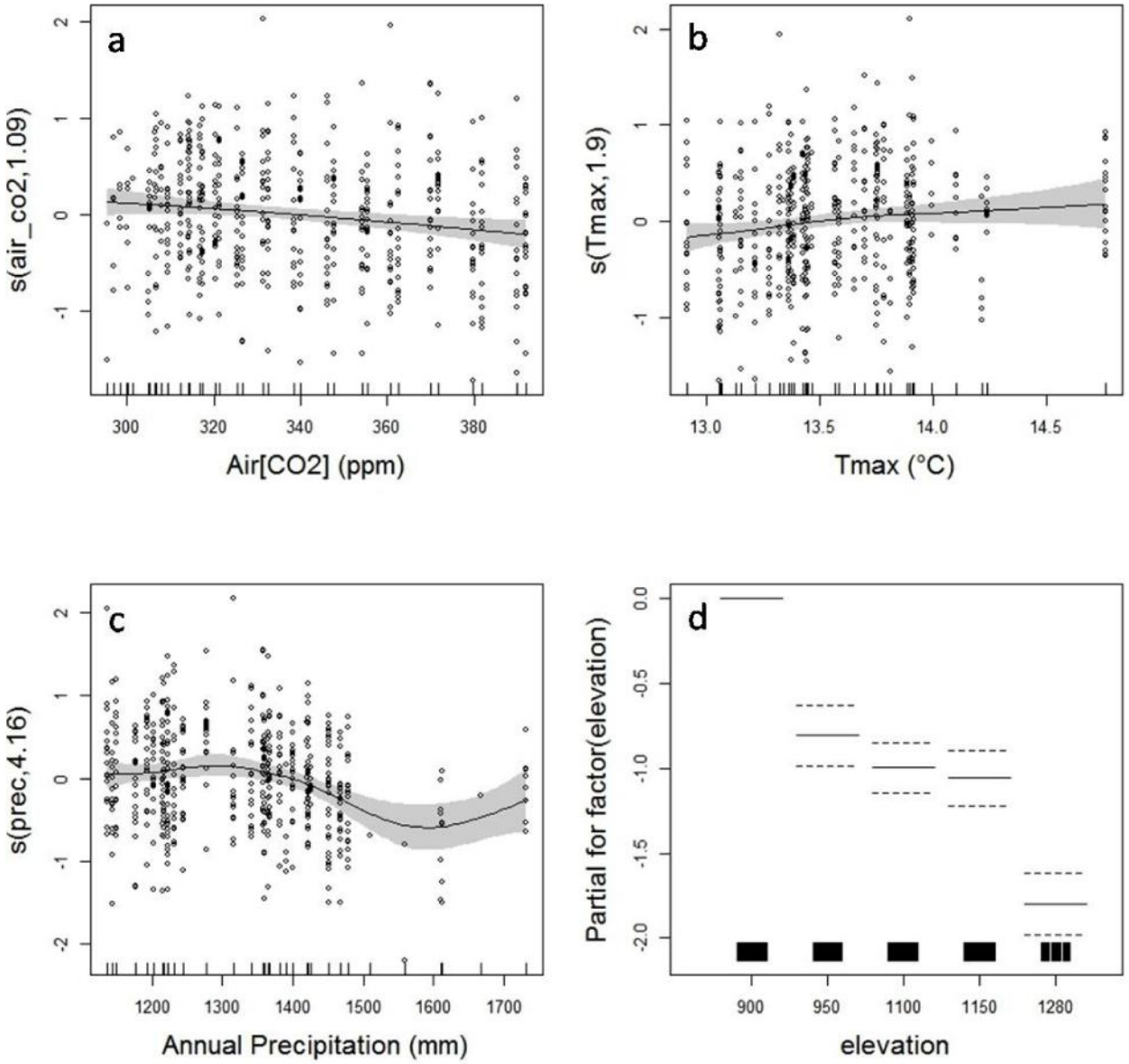


Fig. 10 GAM's results for $\delta^{18}O$. Generalized additive models (GAMs) results show the relationship between $\delta^{18}O$ and environmental and biologic factors. On *y*-axis values indicate the x covariate effect on the tree rings- $\delta^{18}O$ predicted by the model (continuous line) dimensionless and centered around 0, plus the estimated degree of freedom (edf). On *x*-axis each x variable values. Points represent partial residuals from the fitted function and the shaded areas indicate the 95% confidence interval.

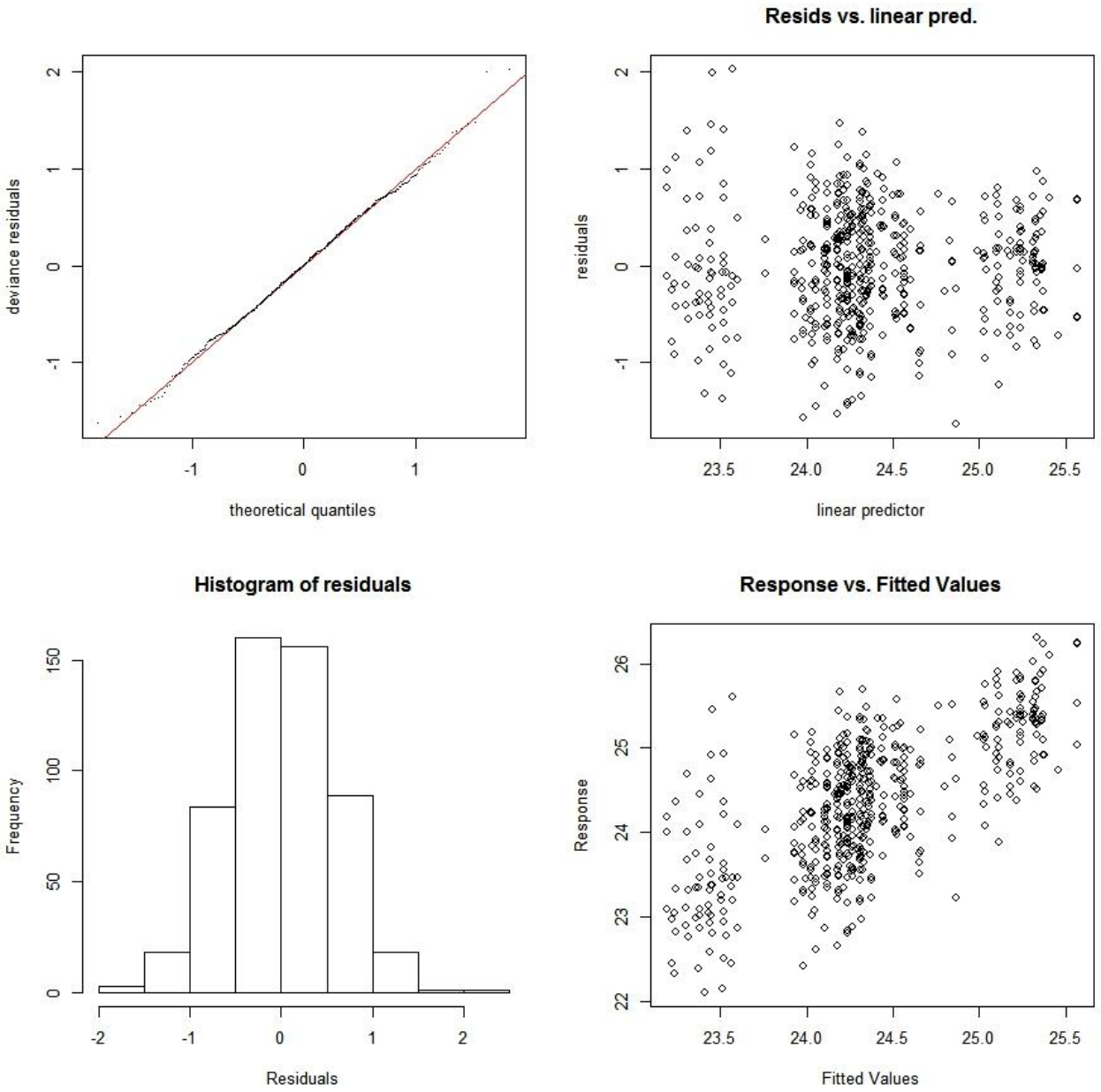


Fig.11 $\delta^{18}O$'s GAMs validation Residual distribution of the whole model and against linear predictor. Response against fitted values for the whole model.

3.4 Dual isotope approach

Finally, the Scheidegger conceptual model was applied in order to disentangle whether stomatal conductance or photosynthetic response has determined the trend in iWUE over the last century, looking at the relationship between $\delta^{18}O$ and $\delta^{13}C$ averaged over all the trees (Fig.12). The pattern between time periods (10 year means) reveals an initial part of the century dominated by a stomatal

response. For example, the 1924-33 and 1944-53 decades, which are characterized by drought crisis events and show a parallel increase in both $\delta^{13}C$ and $\delta^{18}O$, appear to correspond to the c scenario of the Schedigger model (Fig. 1), that is to a marked decrease in g_s without a similar change in A_{max}. In the second part of the century, from the 1954-63 to the 1974-83 decades, the increase in $\delta^{18}O$ without a parallel change in $\delta^{13}C$ would correspond to scenario b, suggesting a strong increase in photosynthetic activity without a parallel change in stomatal conductance. This response appears to come to an end approximately in the first part of the 1980s, and afterwards it changes its direction, suggesting a pronounced reduction in assimilation rate for the 1994-2003 decade (e scenario) possibly associated to the 2003 drought crisis. The last decade unexpectedly shows a parallel decline in both isotopic compositions, corresponding to scenario g of the Scheidegger model, that is to an increase in g_s without a parallel change in A_{max} ; precipitation in the 2004-08 period was above average, although this could have been counter-balanced by the droughts experienced in the following years.

4. Discussion

4.1 Age-related effects

The first aim of this study was to explore the possible influence of a biological (i.e. age/size) effect on isotopic long-term variations. Such an effect could introduce a confounding element when trying to assess the impact of long-term changes in environmental factors affecting tree physiology, or when using stable isotopes for past climate reconstruction (Esper et al. 2010;Brienen et al., 2017). Data analysis showed a strong positive age-related effect on iWUE, while no significant impact was detected in the $\delta^{18}O$ signal. Previous dendroecological studies suggested that an age-related trend is only observed in the early ontogenetic phase of trees' life, defined as juvenile effect (Loader t al. 2007) (Leavitt 2010). This is known to display an increase of about 1.5–2‰ in $\delta^{13}C_p$, normally occurring in the first 40-50 years, implying that no correction for age is needed outside beyond this first interval. The pattern is attributed to enriched $^{13}CO_2$ produced by the respiration of surrounding

dominant trees, and re-absorbed by the young, shaded trees. However, this hypothesis fails to explain our results due to the stand structure (even-aged), and the social position of the sampled trees (always dominant), which were never relegated to the understory layer. Moreover, the age-related effect that is apparent both from a simple synchronic comparison between age classes (Fig. 3) and from the GAMs analysis (Fig. 5) far exceeds the juvenile development stage. More likely, the physiological mechanism implied in this iWUE increase with age-size could be explained if the hydraulic limitation hypothesis (HLH) is taken into consideration (Ryan et al., 1997). The hypothesis suggests a progressive reduction of stomatal conductance as a result of the increased hydraulic resistance of longer stems and branches, as well as the increased gravitational potential opposing the ascent of water in taller trees, and the homeostatic maintenance of a minimum water potential in leaves (Ryan et al., 2006). Such an increase in C isotope discrimination with tree height, beyond the juvenile phase, has been demonstrated for a number of species, including Douglas fir (Mc Dowell *et al.* 2002, Martinez-Vilalta *et al.* 2007). However, assuming that the iWUE trend related to age is influenced by a decline in stomatal conductance, it is surprising not to detect the same effect also in the oxygen isotopic signal, which is well known to be affected by g_s. A possible explanation could be the shift in the $\delta^{18}O$ of soil water as roots become deeper with age. This process of gradually discrimination against $H_2{}^{18}O$, would act in the opposite direction to the pattern associated with the decrease in g_s and could counteract the g_s-dominated age effect, nullifying its trend. The alternative possibility of an age-related increase in iWUE as a result of an increase in A_{max} is not supported by any physiological evidence.

4.2.Environmental and biogeochemical effects

The second objective of the present study was to distinguish, between the main explanatory environmental and biogeochemical variables which have changed over the last century, which ones have determined the overall increase in iWUE observed over the last century, after correcting for age-related effects. As expected, this pattern was found to be directly and linearly related to air

1316 [CO_2] (Saurer et al., 2004;Peñuelas et al., 2011;Keenan et al. 2013), due to the increase in the
1317 photosynthetic substrate available to trees. Interestingly, the rate of N deposition was also found to
1318 be directly related with iWUE, as already shown in other conifers (Guerrieri et al. 2011; Leonardi et
1319 al. 2012), even if in the present study the oxide form alone (NO_y,) was selected by the stepwise
1320 procedure applied. The long-term effect of N deposition on iWUE is understood to be dominated by
1321 its stimulation of A_{max} (Ripullone et al. 2004), which should increase as the N leaf concentration
1322 level (and thus Rubisco and photosynthetic pigments) rise. Such an effect could be caused by both
1323 direct canopy uptake (Nair et al. 2015) and increase of N in the soil, which can promote net
1324 mineralization and consequently, advance the N available to the trees (Aber et al. 2003). The
1325 amount of NO_y deposited at this site, after a strong increase from pre-industrial times until the
1326 1960-1980 period, has shown a reduction in the last 30 years. Remarkably, this trend matches
1327 closely also the observed recent stabilization in iWUE. Hence, taking into consideration also the
1328 results of the dual isotope analysis, which displays a reduction in the A_{max} after roughly the 1980s,
1329 it would appear that a nutritional constraint has determined at least in part the recent variation in
1330 iWUE in Douglas-fir. This hypothesis is consistent with previous findings from controlled FACE
1331 (Free-Air Carbon Enrichment) experiments (Norby et al. 2010) which demonstrated how N
1332 availability could be limiting for tree growth, suppressing the tree response to elevated CO_2.

1333 Finally, the water availability in the previous summer (as captured by the SPEI index) was the other
1334 significant covariate related with iWUE. Its inverse relationship with the dependent variable could
1335 be explained as a consequence of the direct linkage between g_s and water availability. As a rsult,
1336 this forcing of the previous year could influences the canopy status and the leaf area of the current
1337 season (Zweifel et al. 2006).
1338 Coming to consider the factors affecting $\delta^{18}O$, the most relevant seems to be the precipitation effect
1339 (Fig.10c), possibly because of its relationship with air relative humidity and e_a/e_i; the inverse
1340 relationship observed between this covariate and ^{18}O discrimination potentially reflects the increase
1341 of g_s in periods with greater atmospheric moisture (see Eq. 6).

Interestingly, a pronounced altitudinal gradient was also detected in the $\delta^{18}O$ signal (Fig.10d). This could be attributed either to different levels of leaf enrichment, linked to differences in temperature and VPD between plots (Treydte et al. 2014) or to an effect of altitude on source $\delta^{18}O_s$. This would be consistent with the recent observation of an altitudinal pattern in source $\delta^{18}O_s$ on both sides of the Apennine range (Giustini *et al.* 2016)(Fig.13).

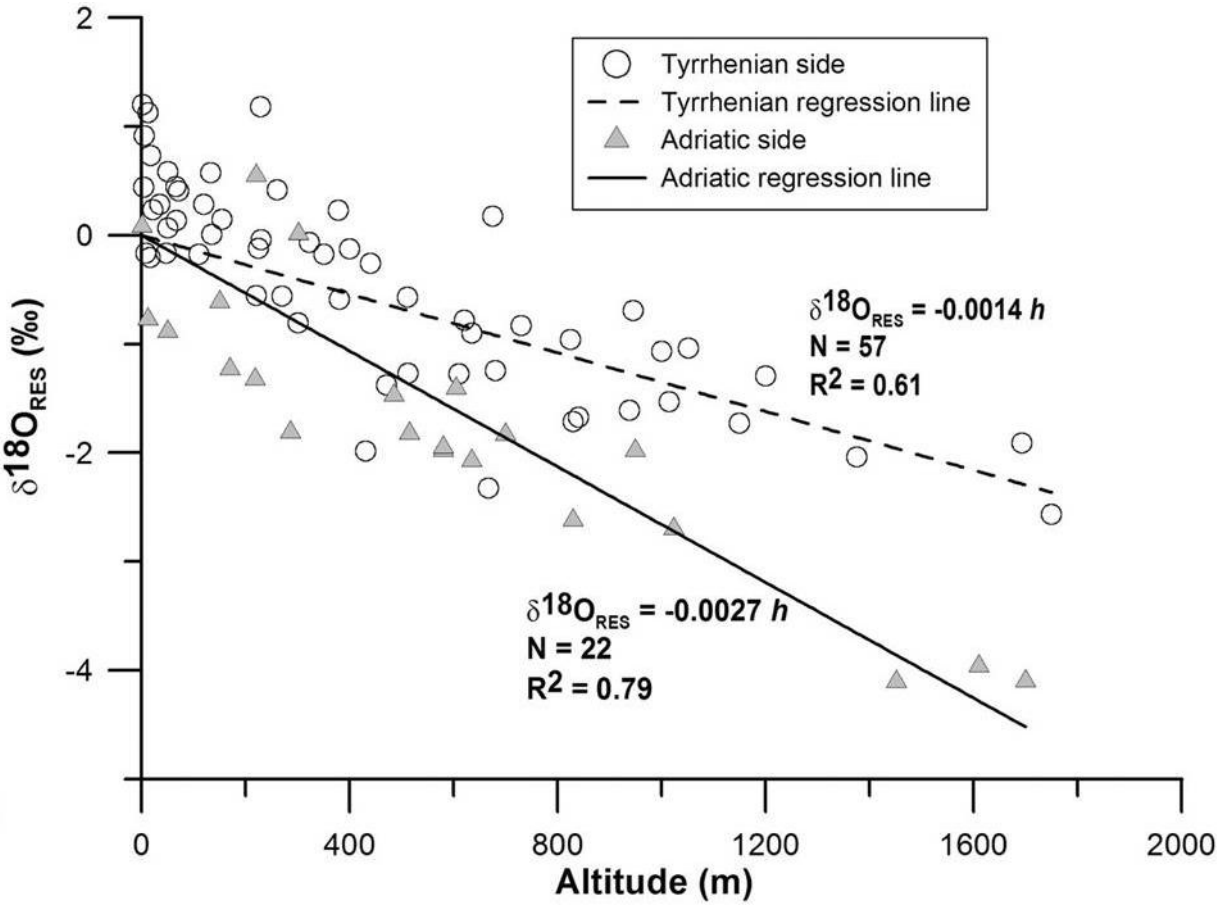


Fig.13 Elevation effect on $\delta^{18}O_s$. Relationship between altitude (m a.s.l.), on the *x*-axis, and $\delta^{18}O$ precipitation residuals, on the *y*-axis for both the Tyrrhenian and the Adriatic side of Italy. From Giustini *et al.* 2016

It seems likely that $\delta^{18}O$ measurements could be substantially affected by the source signal influence, partially hiding the leaf enrichment signal; this source signal influence would be accounted only to some extent by elevation; other significant variables highlighted by the GAMs analysis, such as maximum annual temperature, could also vary locally and be responsible for differences in $\delta^{18}O_s$. Direct measurements of source $\delta^{18}O_s$ could be therefore needed in order to

refine the analysis. A possible solution could be to measure stem water $\delta^{18}O$ as a reference baseline for site-specific differences and to ensure model applicability and interpretation (Roden and Siegwolf 2012).

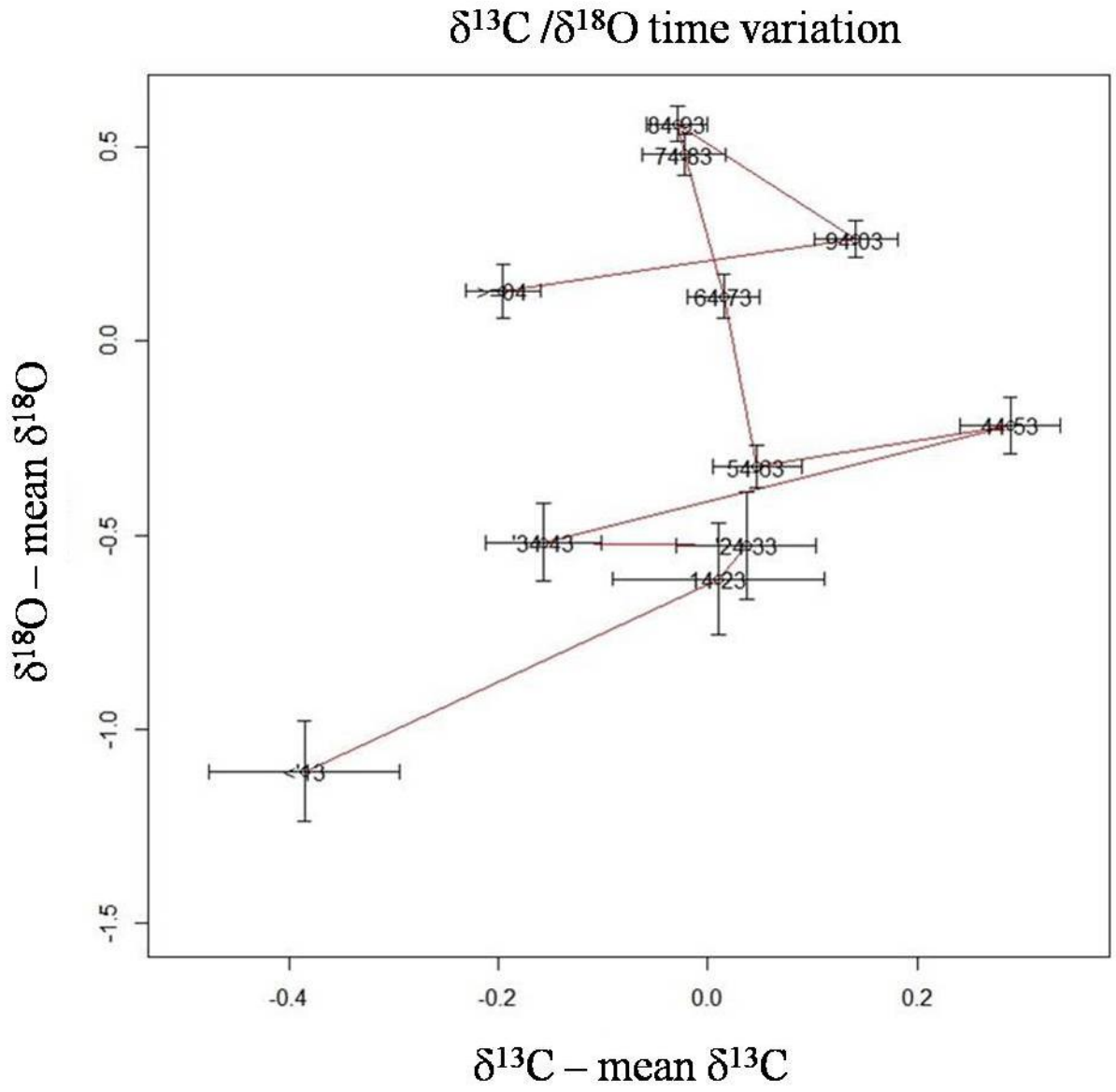


Fig.12 Dual isotope variation over time. Relationship between normalized $\delta^{13}C$, on the *x*-axis, and $\delta^{18}O$, on the *y*-axis. Points are representative of 10-year means of all the trees for the corresponding time period of the last century, as indicated by figures next to the points.

5.Conclusions

Tree ageing appears to result in Douglas fir in an almost linear increase in WUEi, presumably due to the progressive decline in stomatal conductance with increasing height.

Once this effect of age is discounted, global change was found to have induced an initial increase in iWUE during the 1950-1980 period, because of the stimulation of photosynthesis (as demonstrated by the dual-isotope analysis) by the combination of increasing atmospheric [CO_2] and N deposition; this was followed by an apparent saturation over the last decades. This is possibly because of the mismatch between [CO_2] and nutrient availability and the climate-change related increase in transpiration demand, as confirmed by the dual isotope approach application.

Since the analysis of the main factors which have determined the overall increase in iWUE over the last century seems to highlight a multi-factorial control of the physiological response, the use of an additive non-linear model such as GAMs appears to be most appropriate, as it allows to describe such variability, at least partially, and to highlight the long-term influence of each covariate. On the other hand, it should be kept in mind that complex interactions between drivers could lead to misinterpretation in variable selection, due to the additive nature of the model applied.

6.Bibliography

ABER, JOHN D., CHRISTINE L. GOODALE, SCOTT V. OLLINGER, MARIE-LOUISE SMITH, ALISON H. MAGILL, MARY E. MARTIN, RICHARD A. HALLETT, and JOHN L. STODDARD. 2003. “Is Nitrogen Deposition Altering the Nitrogen Status of Northeastern Forests?” *BioScience* 53 (4): 375. doi:10.1641/0006-3568(2003)053[0375:INDATN]2.0.CO;2.

Aho, Ken, DeWayne Derryberry, and Teri Peterson. 2014. “Model Selection for Ecologists: The Worldview of AIC and BIC.” *Ecology* 95 (March): 631–36. doi:10.1890/13-1452.1.

Ainsworth, Elizabeth A., and Stephen P. Long. 2005. “What Have We Learned from 15 Years of Free-Air CO2 Enrichment (FACE)? A Meta-Analytic Review of the Responses of Photosynthesis, Canopy Properties and Plant Production to Rising CO2.” *New Phytologist* 165 (2): 351–71. doi:10.1111/j.1469-8137.2004.01224.x.

Babst, Flurin, M. Ross Alexander, Paul Szejner, Olivier Bouriaud, Stefan Klesse, John Roden, Philippe Ciais, et al. 2014. “A Tree-Ring Perspective on the Terrestrial Carbon Cycle.” *Oecologia* 176 (2): 307–22. doi:10.1007/s00442-014-3031-6.

Beedlow, Peter A., E. Henry Lee, David T. Tingey, Ronald S. Waschmann, and Connie A. Burdick. 2013. “The Importance of Seasonal Temperature and Moisture Patterns on Growth of Douglas-Fir in Western Oregon, USA.” *Agricultural and Forest Meteorology* 169. Elsevier B.V.: 174–85. doi:10.1016/j.agrformet.2012.10.010.

Biondi, Franco. 1999. “Comparing Tree-Ring Chronologies and Repeated timberiInventories as Forest Monitoring Tools.” *Ecological Applications* 9 (1): 216–27. doi:10.1890/1051-0761(1999)009[0216:CTRCAR]2.0.CO;2.

Boettger, Tatjana, Marika Haupt, Kay Knöller, Stephan M. Weise, John S. Waterhouse, Katja T. Rinne, Neil J. Loader, et al. 2007. “Wood Cellulose Preparation Methods and Mass Spectrometric Analyses of ??13C, ??18O, and Nonexchangeable ??2H Values in Cellulose, Sugar, and Starch: An Interlaboratory Comparison.” *Analytical Chemistry* 79 (12): 4603–12. doi:10.1021/ac0700023.

Boisvenue, Céline, and Steven W. Running. 2006. “Impacts of Climate Change on Natural Forest Productivity - Evidence since the Middle of the 20th Century.” *Global Change Biology* 12 (5): 862–82. doi:10.1111/j.1365-2486.2006.01134.x.

Brienen, R J W, E Gloor, S Clerici, R Newton, L Arppe, A Boom, S Bottrell, et al. n.d. “Isotopes.” *Nature Communications*. Springer US, 1–10. doi:10.1038/s41467-017-00225-z.

Brunel, J.P., G.R. Walker, C.D. Walker, J.C. Dighton, and A. Kennett-Smith. 1991. “Using Stable Isotopes of Water to Trace Plant Water Uptake.” *International Symposium on the Use of Stable Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies*, 543–551. doi:10.2144/000114133.

Bunn, Andrew G. 2008. “A Dendrochronology Program Library in R (dplR).” *Dendrochronologia* 26 (2): 115–24. doi:10.1016/j.dendro.2008.01.002.

Camarero, J. Julio, Antonio Gazol, Jacques C. Tardif, and France Conciatori. 2015. “Attributing Forest Responses to Global-Change Drivers: Limited Evidence of a CO2-Fertilization Effect in Iberian Pine Growth.” *Journal of Biogeography* 42 (11): 2220–33. doi:10.1111/jbi.12590.

Carrer, Marco, and C Urbinati. 2006. “Long-Term Change in the Sensitivity of Tree- ring Growth to Climate Forcing in *Larix Decidua*.” *New Phytologist* 170 (iv): 861–72.

Cook, Edward R., Keith R. Briffa, David M Meko, Donald A Graybill, and Gary Funkhouser. 1995. “The ’segment Length Curse’ in Long Tree-Ring Chronology Development for Palaeoclimatic Studies.” *The Holocene* 5 (2): 229–37. doi:10.1177/095968369500500211.

Dawson, T E. 1998. “ Fog in the Californian Redwood Forest: Ecosystem Inputs and Use by Plants.” *Oecologia* 117: 476–85.

Dawson, Todd E., Stefania Mambelli, Agneta H. Plamboeck, Pamela H. Templer, and Kevin P. Tu.

2002. “Stable Isotopes in Plant Ecology.” *Annual Review of Ecology and Systematics* 33 (1): 507–59. doi:10.1146/annurev.ecolsys.33.020602.095451.

Dawson, Todd E., and John S Pate. 1996. “Seasonal Water Uptake and Movement in Root Systems of Australian Phraeatophytic Plants of Dimorphic Root Morphology: A Stable Isotope Investigation.” *Oecologia* 107 (1): 13–20. doi:10.1007/BF00582230.

Di Biase, Giampaolo, Gloria Falsone, Anna Graziani, Gilmo Vianello, and Livia Vittori Antisari. 2015. “Carbon Sequestration in Soils Affected By Douglas Fir Reforestation in Apennines (Northern Italy).” *Eqa-International Journal of Environmental Quality* 17: 1–11. doi:10.6092/issn.2281-4485/5208.

Dongmann, G, and H W Nürnberg. 1974. “On the Enrichment of H2180 in the Leaves of Transpiring Plants I T L Q E” 52: 1–2.

Esper, Jan, Edward R Cook, and Fritz H Schweingruber. 2002. “Low-Frequency Signals in Long Tree-Ring Chronologies for Reconstructing Past Temperature Variability.” *Science (New York, N.Y.)* 295 (5563). American Association for the Advancement of Science: 2250–53. doi:10.1126/science.1066208.

Esper, Jan, David C. Frank, Giovanna Battipaglia, Ulf Büntgen, Christopher Holert, Kerstin Treydte, Rolf Siegwolf, and Matthias Saurer. 2010a. “Low-Frequency Noise in δ13C and δ18O Tree Ring Data: A Case Study of Pinus Uncinata in the Spanish Pyrenees.” *Global Biogeochemical Cycles* 24 (4): 1–11. doi:10.1029/2010GB003772.

Esper, Jan, David C Frank, Giovanna Battipaglia, Ulf Büntgen, Christopher Holert, Kerstin Treydte, Rolf Siegwolf, and Matthias Saurer. 2010b. “Low - Frequency Noise in D 13 C and D 18 O Tree Ring Data : A Case Study of Pinus Uncinata in the Spanish Pyrenees” 24 (2): 1–11. doi:10.1029/2010GB003772.

Farquhar, G. D., L. A. Cernusak, and B. Barnes. 2006. “Heavy Water Fractionation during Transpiration.” *Plant Physiology* 143 (1): 11–18. doi:10.1104/pp.106.093278.

Farquhar, G D, J R Ehleringer, and K T Hubick. 1989. “Carbon Isotope Discrimination and Photosynthesis.” *Annual Review of Plant Physiology and Plant Molecular Biology*. doi:10.1146/annurev.pp.40.060189.002443.

Federal, Swiss, Universitat De Barcelona, J. Julio Camarero, Antonio Gazol, Juan Diego Galván, Gabriel Sangüesa-Barreda, and Emilia Gutiérrez. 2015. “Disparate Effects of Global-Change Drivers on Mountain Conifer Forests: Warming-Induced Growth Enhancement in Young Trees vs. CO 2 Fertilization in Old Trees from Wet Sites.” *Global Change Biology* 21 (2): 738–49. doi:10.1111/gcb.12787.

Fenn, Mark E, Jeremy S Fried, Haiganoush K Preisler, Andrzej Bytnerowicz, Susan Schilling, Sarah Jovan, and Olaf Kuegler. 2015. “REMEASURED FIA PLOTS REVEAL TREE-LEVEL DIAMETER GROWTH AND TREE MORTALITY IMPACTS OF NITROGEN DEPOSITION ON CALIFORNIA ’ S FORESTS” 2013 (Time 2): 2013–16.

Francey, R. J., C. E. Allison, D. M. Etheridge, C. M. Trudinger, I. G. Enting, M. Leuenberger, R. L. Langenfelds, E. Michel, and L. P. Steele. 1999. “A 1000-Year High Precision Record of δ13C in Atmospheric CO2.” *Tellus, Series B: Chemical and Physical Meteorology* 51 (2): 170–93. doi:10.1034/j.1600-0889.1999.t01-1-00005.x.

Frank, D. C., B. Poulter, M. Saurer, J. Esper, C. Huntingford, G. Helle, K. Treydte, et al. 2015.

"Water-Use Efficiency and Transpiration across European Forests during the Anthropocene." *Nature Climate Change*, no. May. doi:10.1038/nclimate2614.

Giustini, Francesca, Mauro Brilli, and Antonio Patera. 2016. "Mapping Oxygen Stable Isotopes of Precipitation in Italy." *Journal of Hydrology: Regional Studies* 8. Elsevier B.V.: 162–81. doi:10.1016/j.ejrh.2016.04.001.

Gómez-Guerrero, Armando, Lucas C.R. R Silva, Miguel Barrera-Reyes, Barbara Kishchuk, Alejandro Velázquez-Martínez, Tomás Martínez-Trinidad, Francisca Ofelia Plascencia-Escalante, and William R. Horwath. 2013. "Growth Decline and Divergent Tree Ring Isotopic Composition (δ13C and δ18O) Contradict Predictions of CO2 Stimulation in High Altitudinal Forests." *Global Change Biology* 19 (6): 1748–58. doi:10.1111/gcb.12170.

Griffin, Daniel, and Kevin J Anchukaitis. 2014. "How Unusual Is the 2012 – 2014 California Drought ?," 9017–23. doi:10.1002/2014GL062433.1.

Guerrieri, R, Maurizio Mencuccini, L J Sheppard, M Saurer, M P Perks, P Levy, M a Sutton, Marco Borghetti, and J Grace. 2011. "The Legacy of Enhanced N and S Deposition as Revealed by the Combined Analysis of Delta 13C, Delta 18O and Delta 15N in Tree Rings." *Global Change Biology* 17 (5): 1946–62. doi:10.1111/j.1365-2486.2010.02362.x.

Harris, I., P. D. Jones, T. J. Osborn, and D. H. Lister. 2014. "Updated High-Resolution Grids of Monthly Climatic Observations - the CRU TS3.10 Dataset." *International Journal of Climatology* 34 (3): 623–42. doi:10.1002/joc.3711.

Hastie, T. J., and R. J. Tibshirani. 1990. "Generalized Additive Models." *Monographs on Statistics and Applied Probability*. doi:10.1016/j.csda.2010.05.004.

IPCC. 2014. "Climate Change 2014 Synthesis Report Summary Chapter for Policymakers." *Ipcc*, 31. doi:10.1017/CBO9781107415324.

Keenan, Trevor F, David Y Hollinger, Gil Bohrer, Danilo Dragoni, J William Munger, Hans Peter Schmid, and Andrew D Richardson. 2013. "Increase in Forest Water-Use Efficiency as Atmospheric Carbon Dioxide Concentrations Rise." *Nature* 499 (7458): 324–27. doi:10.1038/nature12291.

Korner, C. 2005. "Carbon Flux and Growth in Mature Deciduous Forest Trees Exposed to Elevated CO2." *Science* 309 (5739): 1360–62. doi:10.1126/science.1113977.

Leavitt, Steven W. 2010. "Tree-Ring C-H-O Isotope Variability and Sampling." *The Science of the Total Environment* 408 (22): 5244–53. doi:10.1016/j.scitotenv.2010.07.057.

Lee, E. Henry, Peter A. Beedlow, Ronald S. Waschmann, David T. Tingey, Charlotte Wickham, Steve Cline, Michael Bollman, and Cailie Carlile. 2016. "Douglas-Fir Displays a Range of Growth Responses to Temperature, Water, and Swiss Needle Cast in Western Oregon, USA." *Agricultural and Forest Meteorology* 221. Elsevier B.V.: 176–88. doi:10.1016/j.agrformet.2016.02.009.

Leonardi, Stefano, Tiziana Gentilesca, Rossella Guerrieri, Francesco Ripullone, Federico Magnani, Maurizio Mencuccini, Twan V. Noije, and Marco Borghetti. 2012. "Assessing the Effects of Nitrogen Deposition and Climate on Carbon Isotope Discrimination and Intrinsic Water-Use Efficiency of Angiosperm and Conifer Trees under Rising CO 2 Conditions." *Global Change Biology* 18 (9): 2925–44. doi:10.1111/j.1365-2486.2012.02757.x.

Lévesque, Mathieu, Rolf Siegwolf, Matthias Saurer, Britta Eilmann, and Andreas Rigling. 2014. "Increased Water-Use Efficiency Does Not Lead to Enhanced Tree Growth under Xeric and Mesic Conditions." *New Phytologist* 203 (1): 94–109. doi:10.1111/nph.12772.

Linares, Juan Carlos, Jesús Julio Camarero, and José Antonio Carreira. 2010. "Competition Modulates the Adaptation Capacity of Forests to Climatic Stress: Insights from Recent Growth Decline and Death in Relict Stands of the Mediterranean Fir Abies Pinsapo." *Journal of Ecology* 98 (3): 592–603. doi:10.1111/j.1365-2745.2010.01645.x.

Magnani, Federico, Maurizio Mencuccini, Marco Borghetti, Paul Berbigier, Frank Berninger, Sylvain Delzon, Achim Grelle, et al. 2007. "The Human Footprint in the Carbon Cycle of Temperate and Boreal Forests." *Nature* 447 (7146): 848–50. doi:10.1038/nature05847.

Marshall, D D, and R O Curtis. 2002. "Levels-of-Growing-Stock Cooperative Study in Douglas-Fir: Report No. 15 - Hoskins: 1963-1998." *Research Paper Pacific Northwest Research Station, USDA Forest Service*, no. PNW-RP-537: 80.

McCarroll, Danny, and Neil J. Loader. 2004. "Stable Isotopes in Tree Rings." *Quaternary Science Reviews* 23 (7–8): 771–801. doi:10.1016/j.quascirev.2003.06.017.

McMahon, Sean M, Geoffrey G Parker, and Dawn R Miller. 2010. "Evidence for a Recent Increase in Forest Growth." *Proceedings of the National Academy of Sciences of the United States of America* 107 (8): 3611–15. doi:10.1073/pnas.0912376107.

Medlyn, B. E., F. -W. Badeck, D. G. G. De Pury, C. V. M. Barton, M. Broadmeadow, R. Ceulemans, P. De Angelis, et al. 1999. "Effects of Elevated [CO2] on Photosynthesis in European Forest Species: A Meta-Analysis of Model Parameters." *Plant, Cell & Environment* 22 (12): 1475–1495. doi:10.1046/j.1365-3040.1999.00523.x.

Nair, Richard K F, Micheal P Perks, Andrew Weatherall, Elizabeth M Baggs, and Maurizio Mencuccini. 2015. "Does Canopy Nitrogen Uptake Enhance Carbon Sequestration by Trees?" *Global Change Biology*, September. doi:10.1111/gcb.13096.

Nehrbass-Ahles, Christoph, Flurin Babst, Stefan Klesse, Magdalena Nötzli, Olivier Bouriaud, Raphael Neukom, Matthias Dobbertin, and David Frank. 2014. "The Influence of Sampling Design on Tree-Ring-Based Quantification of Forest Growth." *Global Change Biology* 20 (9). doi:10.1111/gcb.12599.

Norby, Richard J, Jeffrey M Warren, Colleen M Iversen, Belinda E Medlyn, and Ross E McMurtrie. 2010. "CO2 Enhancement of Forest Productivity Constrained by Limited Nitrogen Availability." *Proceedings of the National Academy of Sciences of the United States of America* 107 (45): 19368–73. doi:10.1073/pnas.1006463107.

Peñuelas, Josep, Josep G. Canadell, and Romà Ogaya. 2011. "Increased Water-Use Efficiency during the 20th Century Did Not Translate into Enhanced Tree Growth." *Global Ecology and Biogeography* 20 (4): 597–608. doi:10.1111/j.1466-8238.2010.00608.x.

Perakis, Steven S., and Emily R. Sinkhorn. 2011. "Biogeochemistry of a Temperate Forest Nitrogen Gradient." *Ecology* 92 (7): 1481–91. doi:10.1890/10-1642.1.

Peters, Richard L., Peter Groenendijk, Mart Vlam, and Pieter a. Zuidema. 2015. "Detecting Long-Term Growth Trends Using Tree Rings: A Critical Evaluation of Methods." *Global Change Biology*, n/a-n/a. doi:10.1111/gcb.12826.

Phillips, Nathan G., Thomas N. Buckley, and David T. Tissue. 2008. “Capacity of Old Trees to Respond to Environmental Change.” *Journal of Integrative Plant Biology* 50 (11): 1355–64. doi:10.1111/j.1744-7909.2008.00746.x.

Piovesan, Gianluca, Franco Biondi, Aalfredo Di Filippo, Alfredo Alessandrini, and Maurizio Maugeri. 2008. “Drought-Driven Growth Reduction in Old Beech (Fagus Sylvatica L.) Forests of the Central Apennines, Italy.” *Global Change Biology* 14 (6): 1265–81. doi:10.1111/j.1365-2486.2008.01570.x.

Poage, Nathan J, and John C Tappeiner, II. 2002. “Long-Term Patterns of Diameter and Basal Area Growth of Old-Growth Douglas-Fir Trees in Western Oregon.” *Canadian Journal of Forest Research* 32: 1232–43. doi:10.1139/x02-045.

Rehfeldt, Gerald E., Barry C. Jaquish, Javier Lòpez-Upton, Cuauhtémocmoc Sáenz-Romero, J. Bradley St Clair, Laura P. Leites, and Dennis G. Joyce. 2014. “Comparative Genetic Responses to Climate for the Varieties of Pinus Ponderosa and Pseudotsuga Menziesii: Realized Climate Niches.” *Forest Ecology and Management* 324. Elsevier B.V.: 126–37. doi:10.1016/j.foreco.2014.02.035.

Ripullone, Francesco, Marco Lauteri, Giacomo Grassi, Mariana Amato, and Marco Borghetti. 2004. “Variation in Nitrogen Supply Changes Water-Use Efficiency of Pseudotsuga Menziesii and Populus X Euroamericana; a Comparison of Three Approaches to Determine Water-Use Efficiency.” *Tree Physiology* 24 (6): 671–79.

Rita, Angelo, Marco Borghetti, Luigi Todaro, and Antonio Saracino. 2016. “Interpreting the Climatic Effects on Xylem Functional Traits in Two Mediterranean Oak Species: The Role of Extreme Climatic Events.” *Frontiers in Plant Science* 7 (August): 1–11. doi:10.3389/fpls.2016.01126.

Roden, John, and Rolf Siegwolf. 2012. “Is the Dual-Isotope Conceptual Model Fully Operational?” *Tree Physiology* 32 (10): 1179–82. doi:10.1093/treephys/tps099.

Rozanski, Kazimierz, Luis Araguas-araguas, and Roberto Gonfiantini. 2016. “Relation Between Long-Term Trends of Oxygen-18 Isotope Composition of Precipitation and Climate Author (S): Kazimierz Rozanski , Luis Araguás-Araguás and Roberto Gonfiantini Published by : American Association for the Advancement of Science Stable URL :” 258 (5084): 981–85.

Ryan, Mg, D Binkley, and Jh Fownes. 1997. “Age-Related Decline in Forest Productivity: Pattern and Process.” *Advances in Ecological Research*.

Ryan, Michael G., Nathan Phillips, and Barbara J. Bond. 2006. “The Hydraulic Limitation Hypothesis Revisited.” *Plant, Cell and Environment* 29 (3): 367–81. doi:10.1111/j.1365-3040.2005.01478.x.

Ryan, Michael G., and Barbara J. Yoder. 1997. “Hydraulic Limits to Tree Height and Tree Growth.” *BioScience* 47 (4): 235–42. doi:10.2307/1313077.

Saurer, Matthias, Rolf T. W. Siegwolf, and Fritz H. Schweingruber. 2004. “Carbon Isotope Discrimination Indicates Improving Water-Use Efficiency of Trees in Northern Eurasia over the Last 100 Years.” *Global Change Biology* 10 (12): 2109–20. doi:10.1111/j.1365-2486.2004.00869.x.

Scheidegger, Y., M. Saurer, M. Bahn, and R. Siegwolf. 2000. “Linking Stable Oxygen and Carbon Isotopes with Stomatal Conductance and Photosynthetic Capacity: A Conceptual Model.”

Oecologia 125 (3): 350–57. doi:10.1007/s004420000466.

Sitch, Stephan, C. Huntingford, N. Gedney, P. E. Levy, M. Lomas, S. L. Piao, R. Betts, et al. 2008. "Evaluation of the Terrestrial Carbon Cycle, Future Plant Geography and Climate-Carbon Cycle Feedbacks Using Five Dynamic Global Vegetation Models (DGVMs)." *Global Change Biology* 14 (9): 2015–39. doi:10.1111/j.1365-2486.2008.01626.x.

Sternberg, Leonel Da Silveira Lobo O.Reilly. 2009. "Oxygen Stable Isotope Ratios of Tree-Ring Cellulose: The next Phase of Understanding." *New Phytologist* 181 (3): 553–62. doi:10.1111/j.1469-8137.2008.02661.x.

Treydte, Kerstin, Sonja Boda, Elisabeth Graf Pannatier, Patrick Fonti, David Frank, Bastian Ullrich, Matthias Saurer, et al. 2014. "Seasonal Transfer of Oxygen Isotopes from Precipitation and Soil to the Tree Ring : Source Water versus Needle Water Enrichment."

Vicente-Serrano, Sergio M., Santiago Beguería, and Juan I. López-Moreno. 2010. "A Multiscalar Drought Index Sensitive to Global Warming: The Standardized Precipitation Evapotranspiration Index." *Journal of Climate* 23 (7): 1696–1718. doi:10.1175/2009JCLI2909.1.

Vicente-Serrano, Sergio M., J. Julio Camarero, and Cesar Azorin-Molina. 2014. "Diverse Responses of Forest Growth to Drought Time-Scales in the Northern Hemisphere." *Global Ecology and Biogeography* 23 (9): 1019–30. doi:10.1111/geb.12183.

Vitas, A., and K. Žeimavičius. 2006. "Trends of Decline of Douglas Fir in Lithuania : Dendroclimatological Approach." *Baltic Forestry* 12 (2): 200–208.

Walker, Lawrence R., David a. Wardle, Richard D. Bardgett, and Bruce D. Clarkson. 2010. "The Use of Chronosequences in Studies of Ecological Succession and Soil Development." *Journal of Ecology* 98 (4): 725–36. doi:10.1111/j.1365-2745.2010.01664.x.

Warren, Cr, Jf McGrath, and Ma Adams. 2001. "Water Availability and Carbon Isotope Discrimination in Conifers." *Oecologia* 127 (4): 476–86. doi:10.1007/s004420000609.

Williams, David G., R. David Evans, Jason B. West, and James R. Ehleringer. 2007. *Stable Isotopes as Indicators of Ecological Change*. *Terrestrial Ecology*. Vol. 1. doi:10.1016/S1936-7961(07)01024-X.

Wood, Simon N. 2014. "Package ' Mgcv '." doi:10.1186/1471-2105-11-11.Bioconductor.

Wood, Simon N. 2017. "Package ‘ Mgcv .'"

Wood, Simon N. 2006. *Generalized Additive Models: An Introduction with R*. Chapman and Hall/CRC.

Wood, Simon N. 2008. "Fast Stable Direct Fitting and Smoothness Selection for Generalized Additive Models." *Journal of the Royal Statistical Society. Series B: Statistical Methodology* 70 (3): 495–518. doi:10.1111/j.1467-9868.2007.00646.x.

Zhao, Maosheng, and Steven W Running. 2010. "Drought-Induced Reduction in Global Terrestrial Net Primary Production from 2000 Through 2009." *Science* 329 (5994): 940–43. doi:10.1126/science.1192666.

Zweifel, Roman, Lukas Zimmermann, Fabienne Zeugin, and David M. Newbery. 2006. "Intra-

Annual Radial Growth and Water Relations of Trees: Implications towards a Growth Mechanism." *Journal of Experimental Botany* 57 (6): 1445–59. doi:10.1093/jxb/erj125.

Chapter III - Old-growth stand trees reaction to global change, an explorative study

1. Introduction

Over the last 150 years, anthropogenic emissions have dramatically raised the level of [CO_2] in the atmosphere, increased by 45%, from 278 ppm in 1750 to approximately 400 ppm in 2014 (IPCC 2014). The effect of this major forcing of climate change on stem growth in adult trees is still controversial (Korner 2005), as the positive effect of CO_2 could be potentially negated by co-occurring changes in temperature and drought , as well as competition at inter-tree level (Linares et al., 2010). Another potential confounding issues is represented by the difficulty to disentangle biological effects (i.e associated to age/size-related changes taking place over the same time span) from environmental effects (i.e [CO_2], temperature, drought...) when the trees analyzed have been exposd to the external forcing for their entire lifetime (Phillips et al, 2008).

The age-related growth pattern, on the other hand, could be modified by global change itself. It is generally assumed that with increasing age productivity is reduced by a complex interaction of decreasing photosynthetic capacity and increased control of stomata conductance (Ryan et al., 2006), due principally to hydraulic limitations related to age or size. If size and the related constraints, rather than age *per se*, is indeed the main driver which affects carbon gain in old trees (Mencuccini et al. 2005), this implies the possibility that old trees could react to factors which can

improve photosynthetic rates or decrease water constraints responsible for stomatal control (Farquhar et al., 1989); this would suggest the possibility of using old trees and their growth as indicators of global change. On the other hand, radial growth is known to be substantially affected by developmental changes in stand density, which could make it difficult to ascertain the effects of age or global change.

In the Pacific Northwest of the United States, old-growth stands of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco typically display a structure characterized by sparse large trees in the overstory, dominating smaller trees of different sizes and species in the lower and middle canopy layers, with a great amount of dead organic matter, and a patchy distribution of gaps (Spies and Franklin 1991). These old-growth forests show low but highly variable stand densities. For example, in the Oregon Coast Range, stands with a number of trees >100 cm in diameter at breast height (dbh) ranging from 18 to 29 trees/ha and a density of trees >50 cm in dbh of about 39 trees/ha are commonly observed (Spies and Franklin 1991). On the other hand, natural young-growth Douglas-fir stands regenerated after large scale disturbance (logging, wild-fire, wind storm) are reported to have a density of trees >20 cm in dbh of about 363 trees/ha at the age of 50-60, while plantations typically have over 600 trees/ha at the same age (Marshall and Curtis 2002). Due to their complex stand structure and reduced densities in the upper layer, the effects of inter-tree competition could be negligible in old-growth forests, at least in the advanced developmental stages. Furthermore, if the stand is not generated by a large-scale disturbance (where earlier phases can be assimilated to even-aged stands, with locally high densities) also inter-tree competition which affects young individuals, could be less pronounced compared to artificial plantations. In fact, after an initial phase of about 30 years, when intense self-thinning has been reported to reduce the density by an average of 53%, their natural development would lead to growth in less dense conditions (Marshall et al. 1992).

Three possible advantages in the analysis of the effects of global change on trees could be achieved by considering old-growth forest trees.

First, the problem associated with the so-called "segment length curse" (Cook et al. 1995), i.e. the need in dendroclimatological studies to combine together different chronologies derived from living trees as well as preserved wood samples, could be minimized using tree-ring series which span the entire industrial age period; this would make it easier to retain low-frequency signal information, as typically associated with climatic changes.

Second, the analysis of very old trees would make it possible to avoid the use of trees which have lived for their entire lifetime under conditions of elevated [CO2] that have occurred over the last 150 years, making it easier to assess its real influence on growth (Phillips et al., 2008). Finally, dealing with uneven-aged old-growth stands would result in the possibility to deal with a less pronounced effect on growth of competition and stand density, as discussed above, compared to even-aged forest trees.

The aim of the present study is twofold: to evaluate the possibility of separating age -related growth changes from environmentally-driven long-term growth trends superimposed to them, and to understand if Douglas-fir in its native range on the Pacific coast is affected by the changing environmental pressure in a long-term prospective, looking in particular at which variable or combination of variables drives any observed change in growth.

2 Material and methods

2.1 Study area

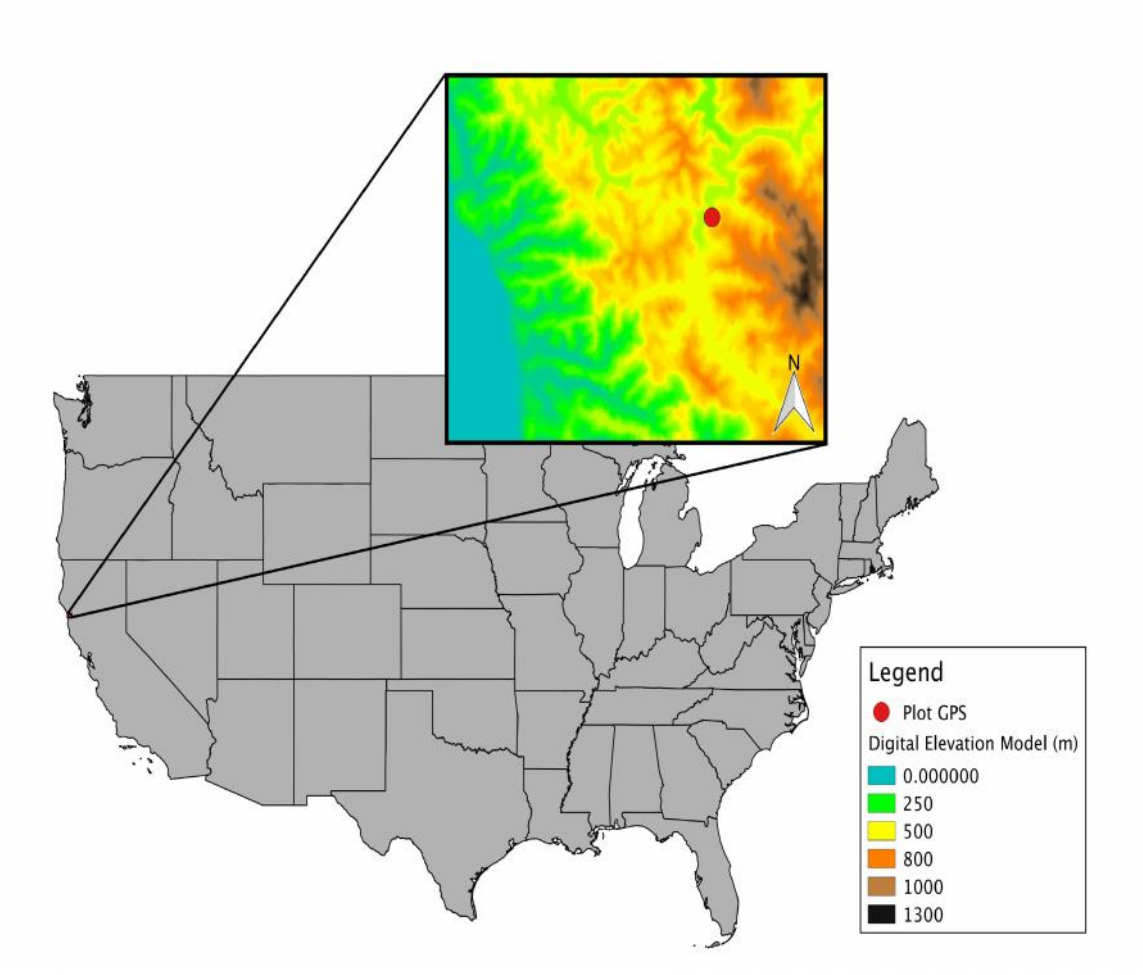


Fig.1 Map shows the location of the old-growth stand sampled (red dot). Different colors are related to different elevations (m, a.s.l).

Tree ring cores originate from a Douglas-fir old-growth stand at about 450-500 m of elevation a.s.l, in the North California Coast Range of Mendocino County. The site is located at 39°43'47.4"N, 123°38'28.2"W, in the University of California Angelo Coast Range Reserve, approximately 250 km north of San Francisco and 20 km east of the Pacificcoast. This region has a Mediterranean

climate with summer droughts, which, especially over the last few years, have reached unprecedented levels in terms of both severity and duration (Griffin and Anchukaitis 2014). Mean annual precipitation is approximately 1350 mm, of which less than 10 mm occurs in the summer months; the summer, however, is characterized by frequent fog presence which is able to mitigate the water evaporative demand (T E Dawson 1998). The mean annual temperature at the site is 12.6°C. The study area is located on a steep north-facing hill slope belonging to the Eldar Creek watershed, and is covered by a mixed forest of Douglas-fir and Mediterranean evergreen species. The species composition is represented by the Pacific Douglas-fir alliance (USDA, 2005), with Douglas-fir as the most represented group associated with Interior Live Oak (*Quercus wislizeni*), Tanoak (*Lithocarpus densiflorus*), and California Bay (*Umbellularia californica*). The forest shows a typical un-even aged structure, with vertical and horizontal spatial complexity, different development stages of living and dead individuals and gaps due probably to small-scale disturbance events. The multiple-layers canopy structure is dominated by older Douglas firs in the over-storey (with heights up to 60-70 m), with low crown closure. An intermediate layer is composed by younger Douglas-firs, while shade-tolerant broad-leaf trees form the lower canopy layer.

2.2 Sampling strategy

The sampling strategy focused on the collection of a representative set of age-classes with the aim to reconstruct a chronosequence; assuming a strong relationship between age and height, sampled trees were therefore chosen on the basis of individual height classes, identified trough a digital canopy height model derived from multiple LiDAR overflights (Fig.1), . A regression among the circumference of sampled trees and their presumptive height as determined from LiDAR maps was subsequently computed, so as to check the goodness of the tree selection approach (Fig. 2). Younger individuals were collected in medium-sized gaps, less than 50 m in diameter, originated

probably from a single disturbance event and covered by a tree cohort with an even-aged structure. Older trees were sampled far from currently evident competitors.

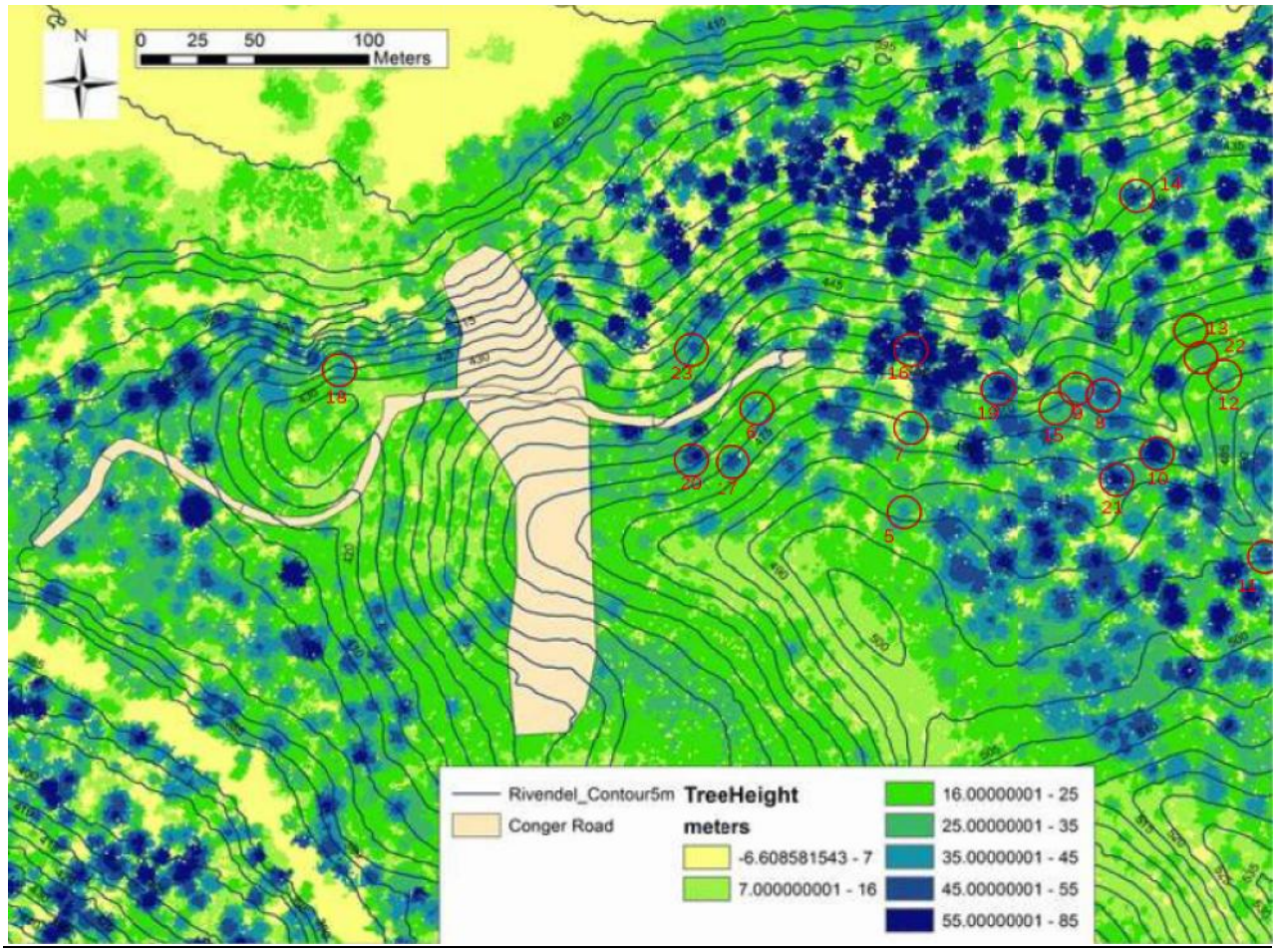
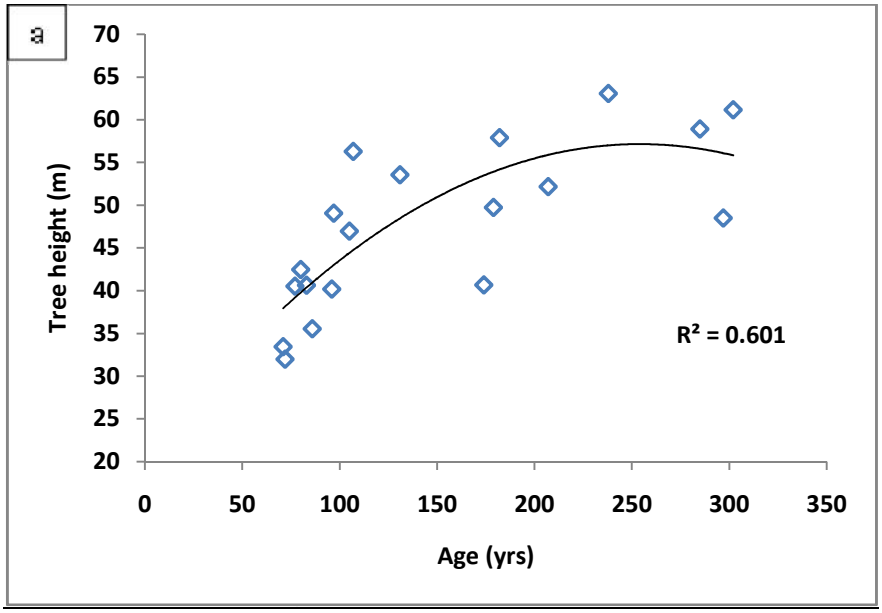


Fig. 1. Site LIDAR image. Image derived from LIDAR flight data; the red circles correspond to the sampled trees. Different colours refer to differents height classes (expressed in meters, in the legend).



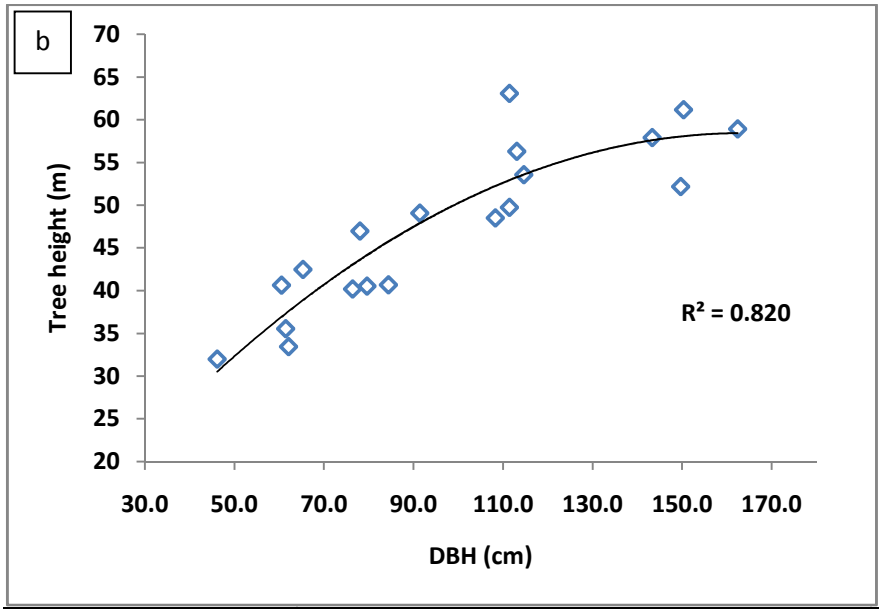


Fig. 2 Relationship between tree height, diameter and age. a. Relationship between height of sampled trees, based on LIDAR data, and (a) tree age, as reconstructed from tree ring data, or (b) diameter at breast height (DBH), based on direct measurements. A 2-nd order polynomial curve has been fitted to the data.

2.3 Tree-ring data

In the fall of 2014, 18 dominant trees were sampled for dendro-ecological analysis. One single core was extracted from each tree with a 5.1 mm Pressler borer (Haglöf, Sweden) at breast height from

the upslope side of trees. The extracted cores were then air-dried and polished with progressively finer sandpaper (60- to 300-grit), so as to distinguish annual ring boundaries. Ring width series were measured on pictures taken with a high definition flatbed scanner (Epson V550 Photo, US), with a precision of 2400 dpi (dots per inch). Measurements were performed with COORECORDER image software analyzer (CybisElektronikand Data AB) with a 0.01 mm accuracy. Samples were visually cross-dated against a reference curve, between and within the series using a correlation coefficient, Gleichläufigkeit values and Student's t-test as indices. Since it was not possible to find a comparable reference curve in the International Tree-Ring Data Bank (TRDB), the reference curve was developed from the dataset itself, by a 'leave-one-out' approach, starting from samples which show a higher correlation between each other. Subsequently, the quality of cross-dating was checked and cross-correlation analysis was performed using CDENDRO software (Cybis Elektronik and Data AB) and the R dplR package (Bunn, 2010). Where the extracted core did not reach the pith of the tree, the length to the center was estimated using the curvature of the last complete ring, and the number of missing rings was calculated by dividing this distance by the last five-year ring average (Applequist et al., 1958). This made it possible to reconstruct the age of each sampled tree.. Subsequently, the raw ring widths recorded were converted into basal area increments (BAI), as the latter allows to compensate for the age effect purely associated with stem geometry, especially relevant at young age, but preserving the low frequency variability (Biondi 1999). Moreover, basal area increments are considered a better proxy of tree volume growth compared with radial increments. Basal area increments were calculated as:

$$BAI = \pi (r^2_t - r^2_{t-1}) \quad (1)$$

where r_t is the stem radius at a given year, and r_{t-1} corresponds to the radius in the previous year.

A preliminary analysis demonstrated a lack of homoscedasticity in the data, i.e. an increase in data variance with increasing BAI. All further statistical analyses were therefore performed on log-transformed BAI (Camarero et al. 2015), although at the cost of making the interpretation of results

1791 less straightforward.

1792

1793 ## 2.4 Climate data

1794 Climatic and geochemical data needed for growth change attribution were not directly masured at
1795 the study site, but derived from available datasets for the period 1901-2014.
1796 Average monthly temperatures and precipitation for the period were obtained, using interpolated
1797 climatic data at gridded 0.5° x 0.5° resolution, from the CRU (Climatic Research Unit of University
1798 of East Anglia, Norwich, UK) TS 4.1 data set (Harris et al. 2014); mean annual temperatures (T_m)
1799 and total annual sums of precipitation (P) were derived from monthly data. To evaluate the potential
1800 effect of drought stress, the Standardized Precipitation Evapotranspiration Index (SPEI) was
1801 adopted and calculated as in Vicente-Serrano et al. (2014). The 1–24 month timescale SPEI values
1802 were computed for each month (Fig.3) and a single representative value was retained for further
1803 analyses (Fig.4), based on Pearson's correlation coefficient.
1804

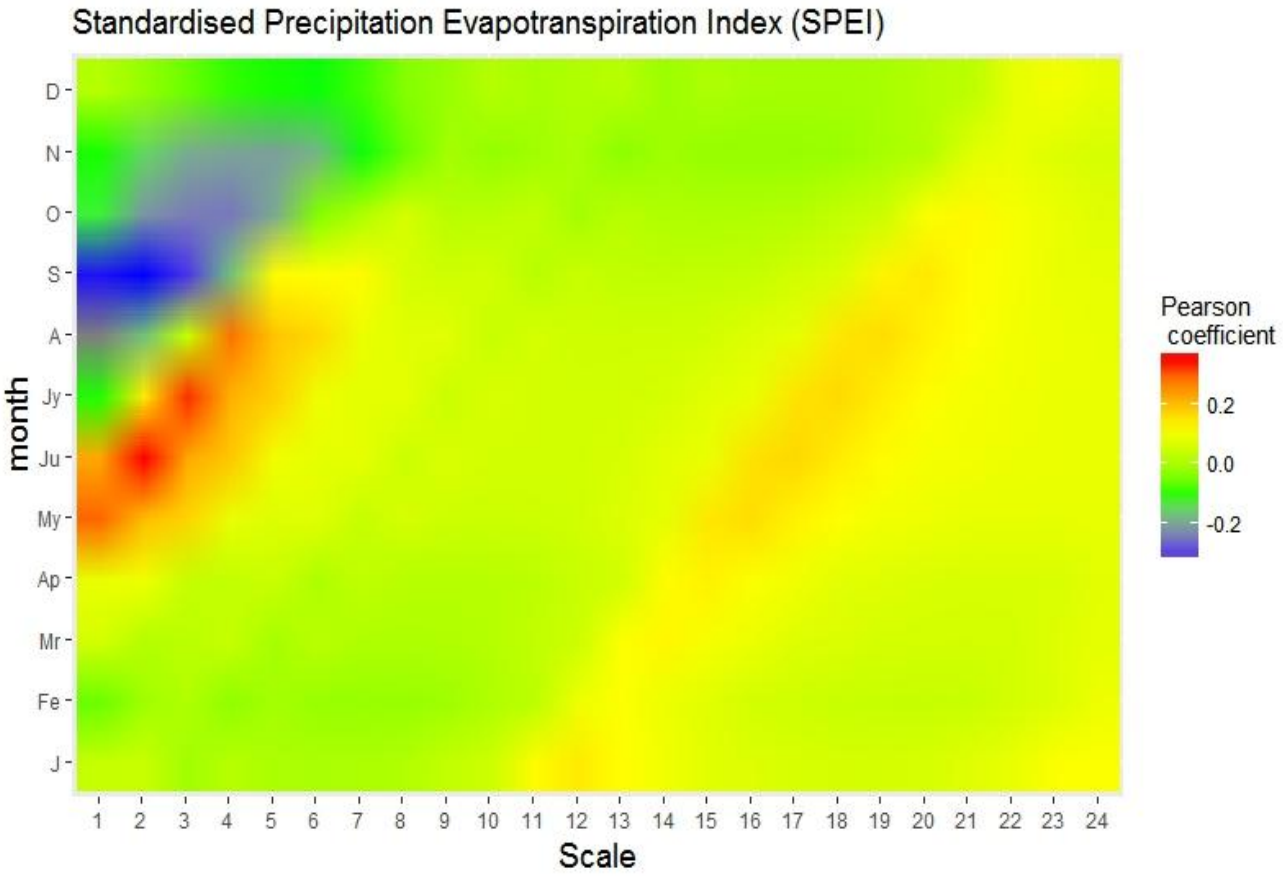


Fig.3 SPEI correlation RWI heatmap. Correlations (Pearson coefficient) between the Standardized Precipitation Evapotranspiration Index (SPEI) at 1- to 24 month scales, and de-trended tree-rings index (RWI), with on the *x*-axis temporal scale of SPEI and on the *y*-axis related months.

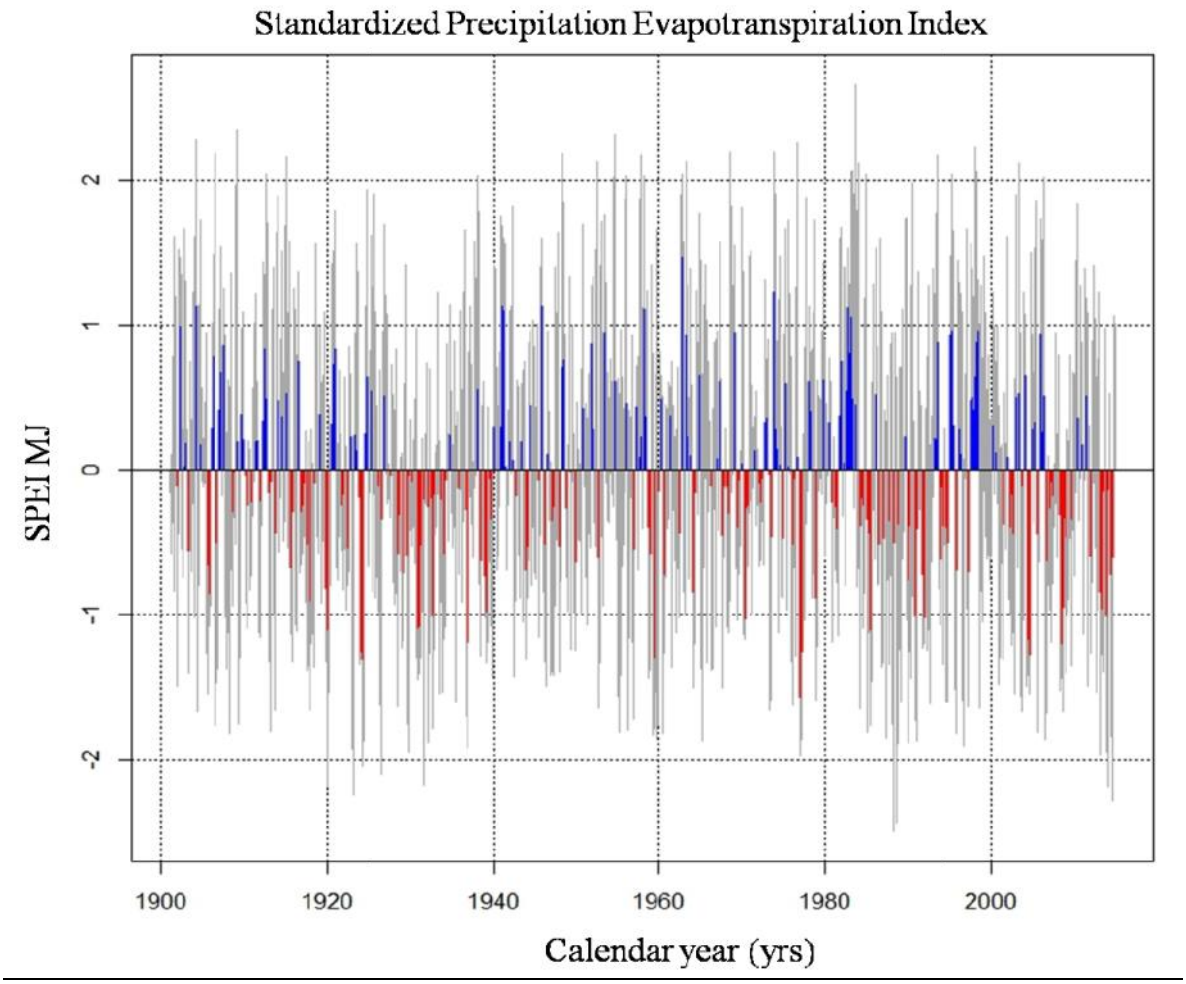


Fig.4 SPEI MJ. Trend of June SPEI at 2 month scales (May,June),which displays the highest correlation with RWI, with on the *x*-calendar year (yrs) and on the *y*-axis SPEI values centered around 0. Red bar represent water deficit, while blue bars represent water surplus.

2.2 Geochemical data

Mean annual data for air CO_2 concentration were obtained from the NOAA Earth System Research Laboratory, as recorded at the Mauna Loa observatory in Hawaii from 1959 to present, and further integrated with the historical dataset proposed by McCarroll and Loader (2004) for the previous priod (1901-1958). Average annual values of nitrogen oxide (NOy) and ammonium (NHx) species for both dry and wet atmospheric deposition were extracted from the global NCAR data set managed by the IGAC-SPARC CCMI (Chemistry-Climate Model Initiative), (available for download at http://blogs.reading.ac.uk/ccmi/). These N depositions data were generated with the NCAR (National Center for Atmospheric Research) atmospheric transport model, covering the

period between 1901 and 2014, which provides gridded (resolution of 2.0°x 2.25°, longitude x latitude) temporal simulation of the chemical composition of the atmosphere.

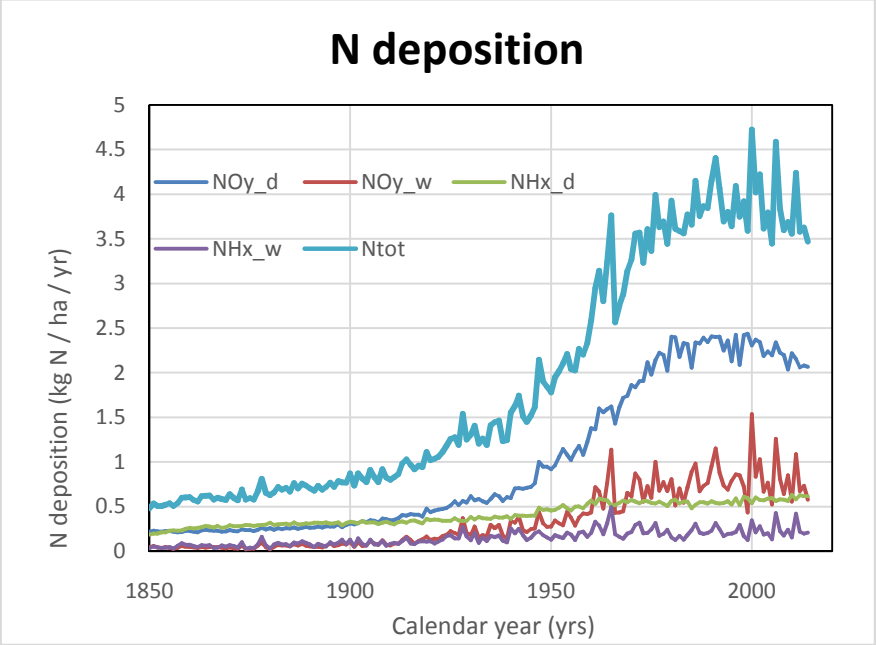


Fig 2. N deposition. Nitrogen deposition trends at Vallombrosa site as modeled by NCAR. Different colors represent the different species (oxide or ammonium) and different form of deposition (wet or dry) plus the total. On the *x*-axis calendar year (yrs), on the *y*-amount of deposition (kg N/ha/yr).

2.3 GAMs

In order to take into account the possibility of non-linear responses to both biological and environmental factors, and to prevent the loss of low-frequency variability potentially associated with traditional de-trending methods, GAMs regression techniques (Hastie and Tibshirani 1990) were applied to the data in the form:

$$y_i = \alpha + f_1(x_{i1}) + \cdots + f_n(x_{in}) + \varepsilon_i \qquad \text{where } \varepsilon i \sim N(0,\sigma^2)$$

where y_i is the i-th value of the response variable, α is the unknown intercept of fixed parameters, $x_1,\ldots,x_n$ are independent variables, $f_1,\ldots,fn$ are smooth functions and εi are residuals with normal (Gaussian) distribution and constant variance. A cubic penalized spline was used as a smooth function, with the amount of penalizations automatically computed by the maximum likelihood (ML) estimation method (S. N. Wood 2006).

As a first step, the approach was used to model the effects of cambial age and time (i.e. global change) on log-transformed basal area increments. As a second step, the GAM approach was applied to try and partition the effects of global change to its climatic and geochemical components. Tree age, atmospheric [CO_2], total atmospheric N deposition or its NH_x and NO_y components, mean (T_m) or maximum (T_{max}) and minimum (T_{min}) annual temperatures, annual precipitation (P) and the SPEI value of the current and previous year ($SPEI_{t-1}$) were considered as possible covariates.

Potential covariates considered in the analysis are cambial age, atmospheric [CO_2], total N atmospheric deposition (N_{tot}, both dry and wet), its and its ammonium species (NH_x) or N oxide species componnt (NO_y), mean (T_m), maximum (T_{max}) and minimum annual temperature (T_{min}), annual precipitation (P), and the selected value of SPEI of the current year (SPEI) and the previous year ($SPEI_{t-1}$). The covariates selection was performed by a stepwise backward process based on p-values for candidate removal.

3. Results and discussion

The different age classes showed extremely different values in terms of raw basal area increments, both along calendar year and by cambial age. From a synchronic perspective (i.e. comparing values corresponding to the same calendar year in the three age classes; Fig. 3), the growth rate of old trees over the last few years appears to be much lower than for younger age classes; this contrasts, however, with the historic growth pattern of the old class, which shows a stabilization in its growth trend after an initial culmination, and only a bland increase in recent decades. The BAI trend of the youngest class shows a steep increase immediately after the 1960s, while the middle age-class shows a more gradual increase over the entire period.

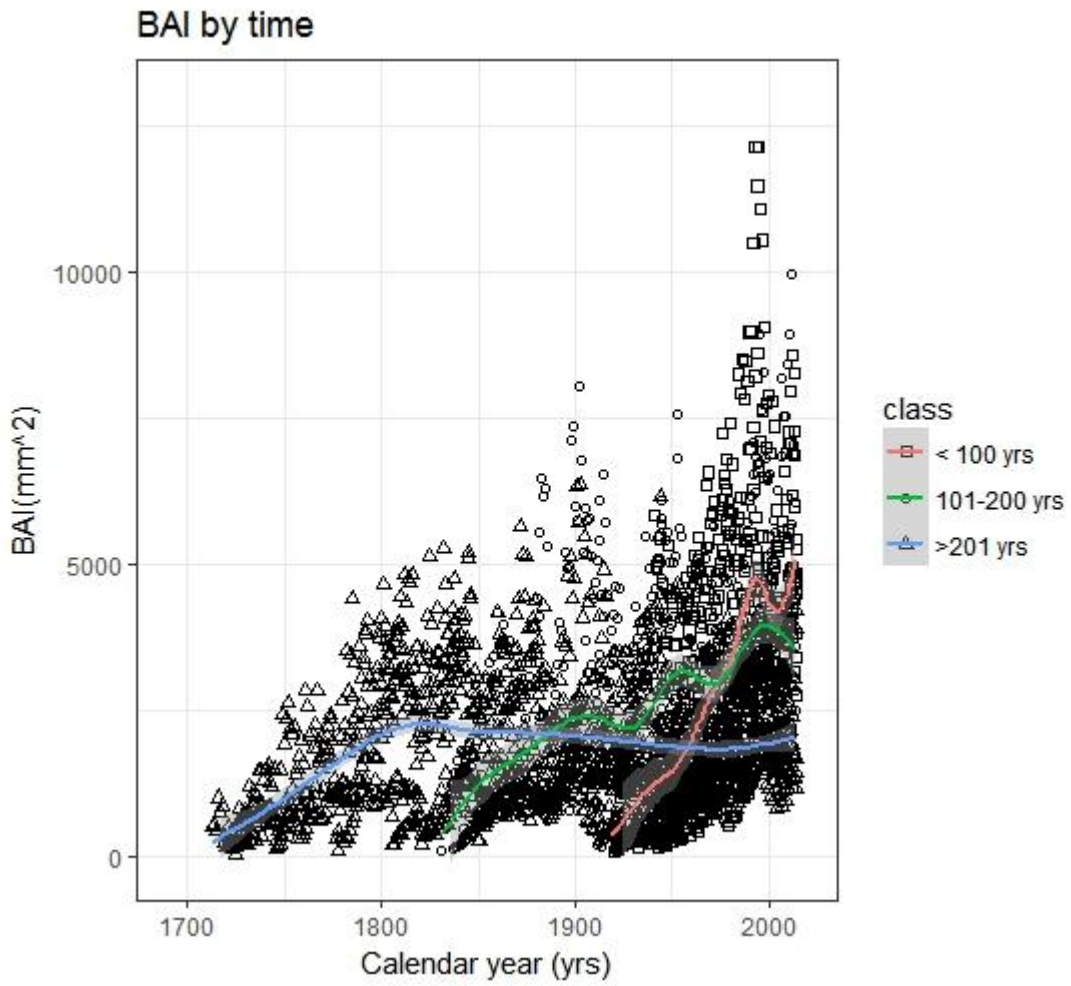


Fig. 3 Time-related dynamics of basal area increments in different age-classes. Time series of basal area increments (BAI), grouped by age-class and fitted with a cubic spline. The shaded areas indicate the 95% prediction interval of the function.

A more consistent picture emerges when taking a diachronic perspective (i.e. comparing values aligned by cambial age; Fig. 4): in this case, the distribution of BAI for the three age-classes seems to follow a gradient, with the younger class exhibiting significantly higher values in comparison with the older one, and the middle-age class displaying an intermediate pattern. Apart from absolute values, there appears to be also a shift in age-related dynamics, with an earlier culmination in the young age class than in the middle-aged and, to an even larger extent, the oldest class. In combination, these features suggest a progressive increase in yield class over time, possibly as a result of a time dependent forcing (i.e global change).

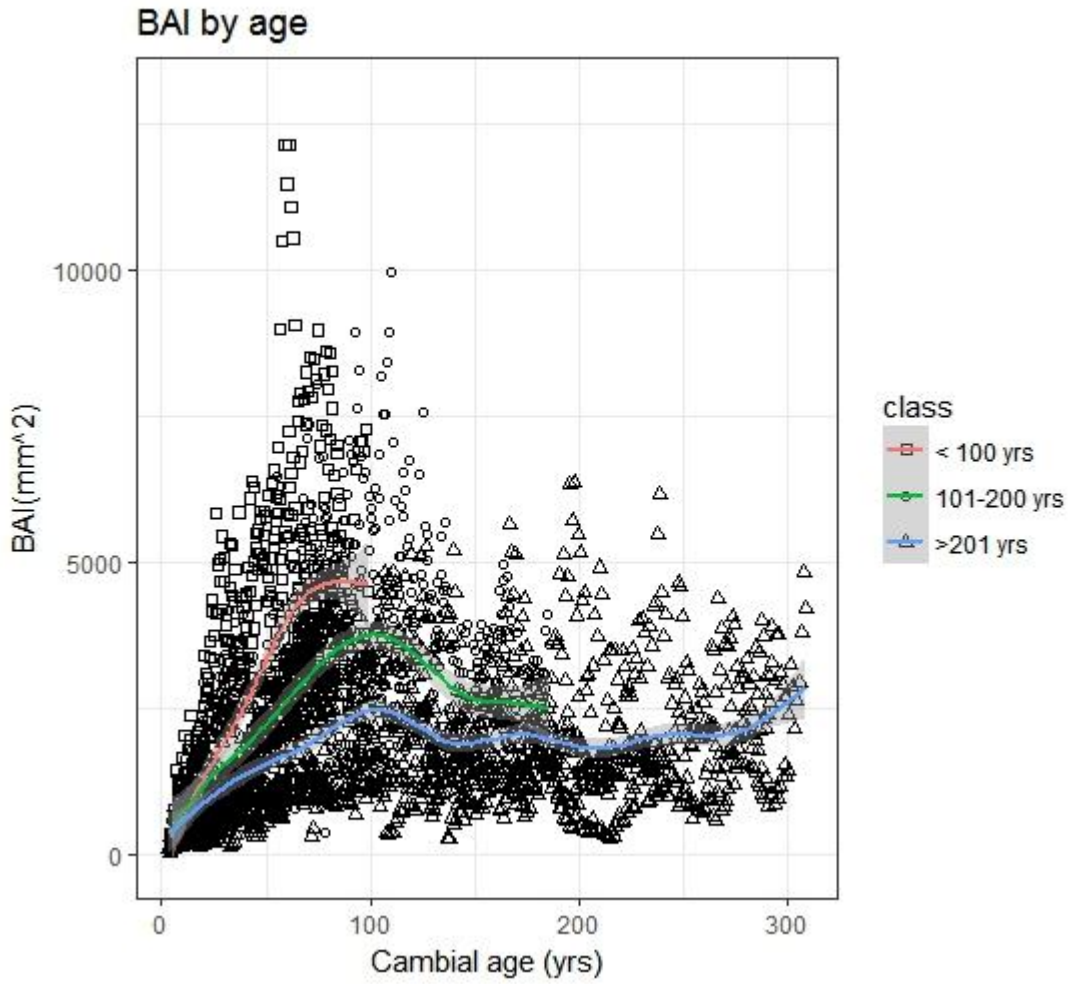


Fig. 4 Diachronic analysis of age effects on basal area increments in different age-classes. Time series of basal area increments (BAI), grouped by age-classes and fitted with a cubic spline. The shaded areas indicate the 95% prediction interval of the spline function

At the same time, the possibility that the pattern could be the result of a biased sampling strategy should be taken into account. On the one hand, the lack of a truly random selection of trees (Nehrbass-Ahles et al. 2014)could have caused an unrepresentative sampling of the entire population; on the other hand, we cannot rule out the possibility that the older age classes are more represented by slow-growing trees, which have greater surviving rate (Issartel and Coffard 2011; but see Kaufmann 1996), while younger class could be represented by a higher fraction of fast-growing trees, leading to an apparent increase of growth rates in time (Peters et al. 2015). Another criticism concerns the ntire dataset and is represented by the strength of the common signal shared by all series. While mean series inter-correlation (SI) is about 0.5 (Table 2), the global expressed

population signal is 0.713, which is below the conventional threshold of 0.85 used to define the acceptability of a tree ring chronology (Cook 1985; Mérian and Lebourgeois 2011). However the running EPS (Fig. 5) shows that, in the more recent period where the growth/environmental analysis was carried out (1901-2014), the EPS reaches a value close to the acceptable threshold. The limited number of replications of this explorative study, of course, affects the strength of the signal, suggesting that results should be considered with care.

Tab.1 Descriptive statistics for raw tree ring width (TRW) and ring width index (RWI) chronologies of the different age-classes. *Mage* in the mean age of the class*, MW* is mean ring width, *SD* is standard deviation of ring width, *MS* is mean sensitivity, *AR1* the first order autocorrelation, ESP the expressed population signal, *SI* the series inter-correlation

		TRW				RWI	
Age-class	*Mage*	*MW*	*SD*	*MS*	*AR1*	*ESP*	*SI*
100	81	3.1030	0.8265	0.1518	0.6853		
200	154	2.2798	0.8340	0.1684	0.7776		
300	267	1.4186	0.8032	0.1834	0.8524		
total	153	2.2671	0.8212	0.1679	0.7718	0.713	0.5

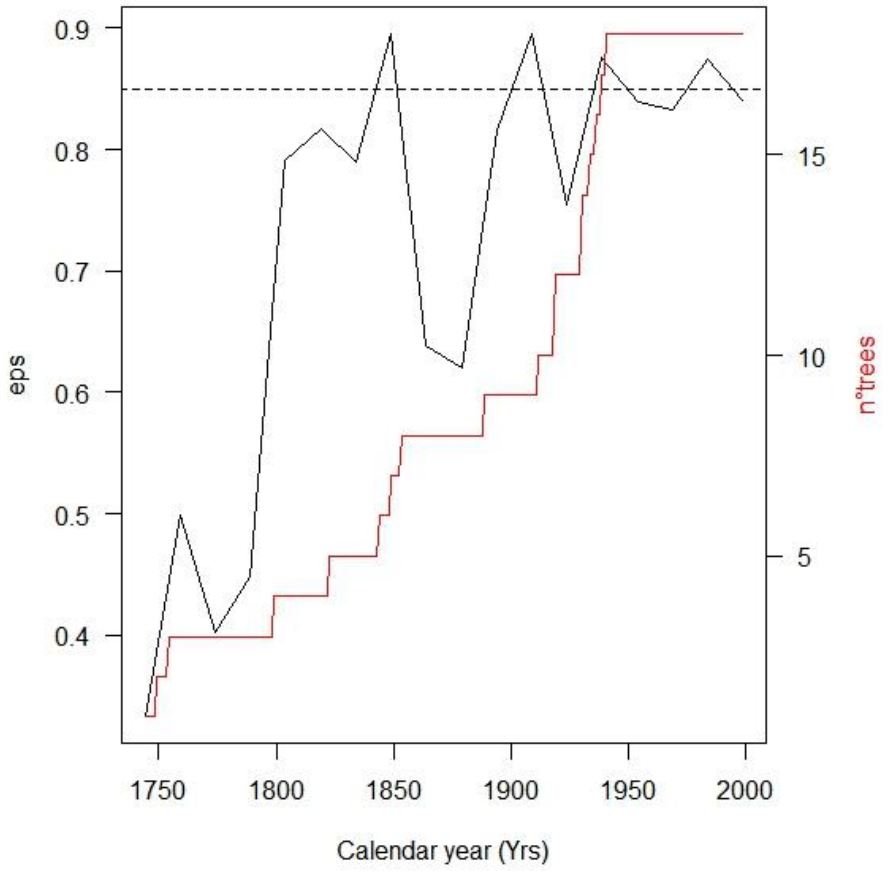


Fig. 5. Preliminary analysis of chronology sample size. Expressed population signal (EPS, dimensionless; black line and left axis) changes along the ring width chronology. The sample size of the chronology, expressed as number of trees as a function of date, is also presented (red line and right axis). The dashed line represents the threshold of 0.85, used to define the acceptability of the chronology.

In order to better understand the possible change of growth rates over time, and separate it from the co-occurring effects of age, the transformed BAI signal was first modeled by the following GAMs model:

$$\text{logBAI} = s(\text{Age}) + s(\text{TIME}) + \varepsilon_i \quad (3)$$

where *s(*Age) is the cambial age effect and *s*(TIME) represents all of the environmental effects cumulated into a single global variable, varying over time, which could be associated with a global change effect. The age-related signal (Fig. 6a) shows a culmination in increments approximately between an age of 80-100 years, followed by a constant decrease to an age of about 200 years, and a

stabilization afterwards with only minor oscillation; a similar pattern would be expected in the age-dependent dynamics of BAI in dominant trees (Poage and Tappeiner 2002). On other hand, the BAI global long-term trend (Fig. 6b), after the subtraction of the age-related signal, displays a pronounced increase in the second part of the 20th century. This trend amounts to an increase of about 30.7% in the period between 1810 (before the beginning of the industrial revolution) and 1950 (before modern industrialization). Afterwards, a 59.5% increase was can be observed to the present, with an accelerating trend that would appear to mirror the recent rise in atmospheric CO_2 concentration.

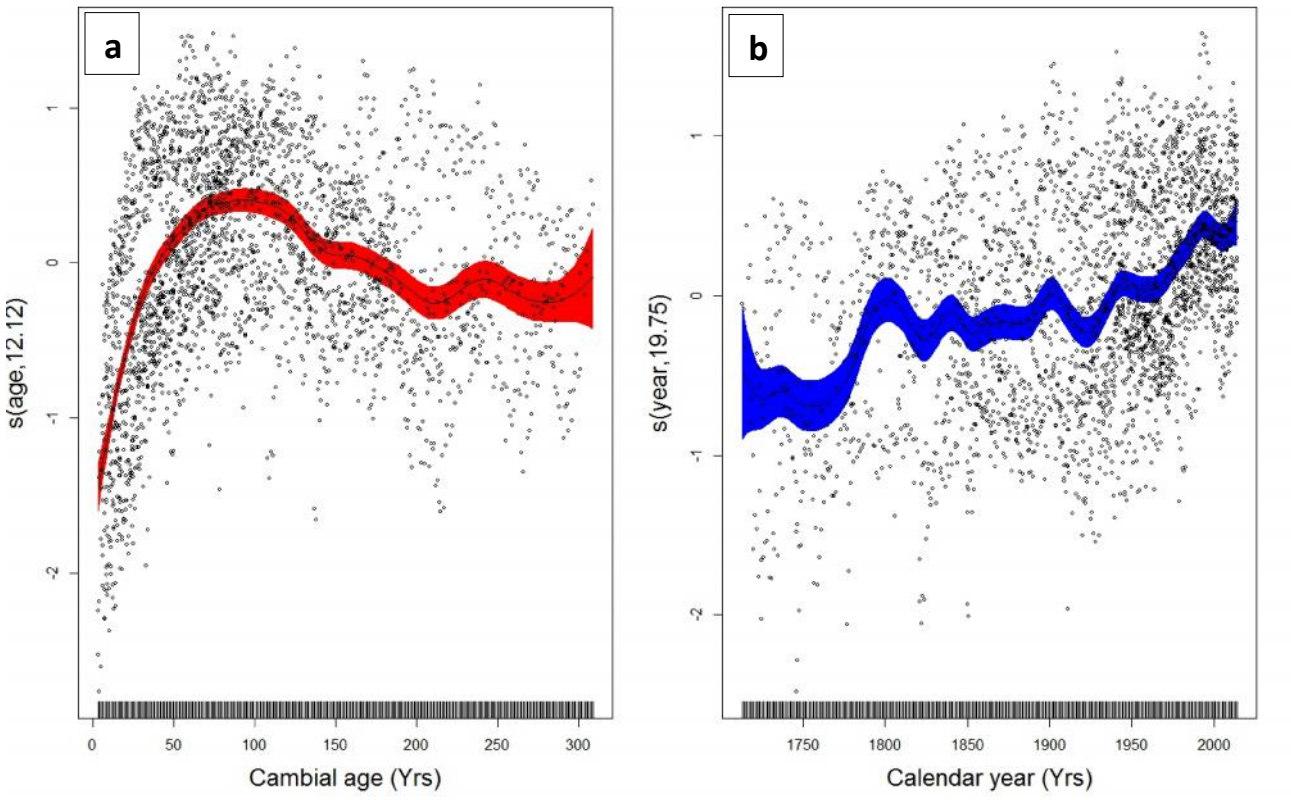


Fig. 6 GAM analysis of the independent effects on BAI of age and time. **a.** Trend of basal area increments (BAI) as a function of age , after correcting for time-related effects. On *x*-axis age (years), and on *y*-axis the function of age *f*(AGE), dimensionless and centered around 0. **b.** Global trend of BAI as a function of time , after correcting for age-related effects. On *y*-axis the function of time *s*(TIME), dimensionless and centered around 0. Points represent partial residuals from the fitted function and the shaded areas indicate the 95% prediction interval of fitted adaptive splines. The GAM model was applied to log-transformed BAI data, so as to correct for heteroscedasticity.

As a last step, in order to highlight which factors were responsible for such a growth acceleration, climatic and geochemical variables were added to the model instead of the time variable. After a backward stepwise selection of candidate covariates, the resulting optimal model was specified as follow (Fig. 7):

$$\log(\mathrm{BAI}) = s(\mathrm{Age}) + s(\mathrm{CO_2}) + s(\mathrm{SPEI\ MJ}) + \varepsilon_i \quad (4)$$

where s(Age) is the cambial age effect, CO_2 is the annual level of atmospheric [CO_2], and SPEI MJ represents the June SPEI values cumulated over the preceding 2-month period. All three effects show a significant p-value at 0.001 level (Table 3) and the global adjusted R^2 for the whole model is 0.462. Atmosphric CO_2 concentrations, rather than climatic covariates, seem to be the major forcing which has determined the long-term trend in Douglas-fir's radial growth in this old-growth forest. Even if early summer water availability shows a significant effect, this is rather weak compared to the geochemical variable. This could be related to the mitigating action of fog and to the changing magnitude and inconstant time-scale of recurring drought events, although it should be considered one of the main limiting factors in this sub-Mediterranean environment. This results makes sense if SPEI is more related with high frequency variability and, thus, plays a secondary role compared to the stronger increase of [CO2]. In fact no clear trend are displayed by none of the climate covariates in the last century (data not shown).

Tab. 2| Generalized additive model results. Relationship between ln(BAI) series (as dependent variable) in *Pseudotsuga menziesii* and environmental or biological factors remaining after the backward selection procedure: cambial age, atmospheric [CO2] and Standardizd Precipitation Evapotranspiration Index computed over May and June (SPEI MJ). *e.d.f.* are effective degree of freedom, *F* is the F-test for variance explained, *P* is the p-value and $R^2(adj)$ is the adjusted correlation coefficient of the model.

Factor	*e.d.f.*	*F*	*P*	$R^2(adj)$
Age	8.131	104.347	< 2e-16	
CO2	3.987	14.201	< 2e-16	
SPEI MJ	1.258	1.807	2.47E-05	
Whole model				0.462

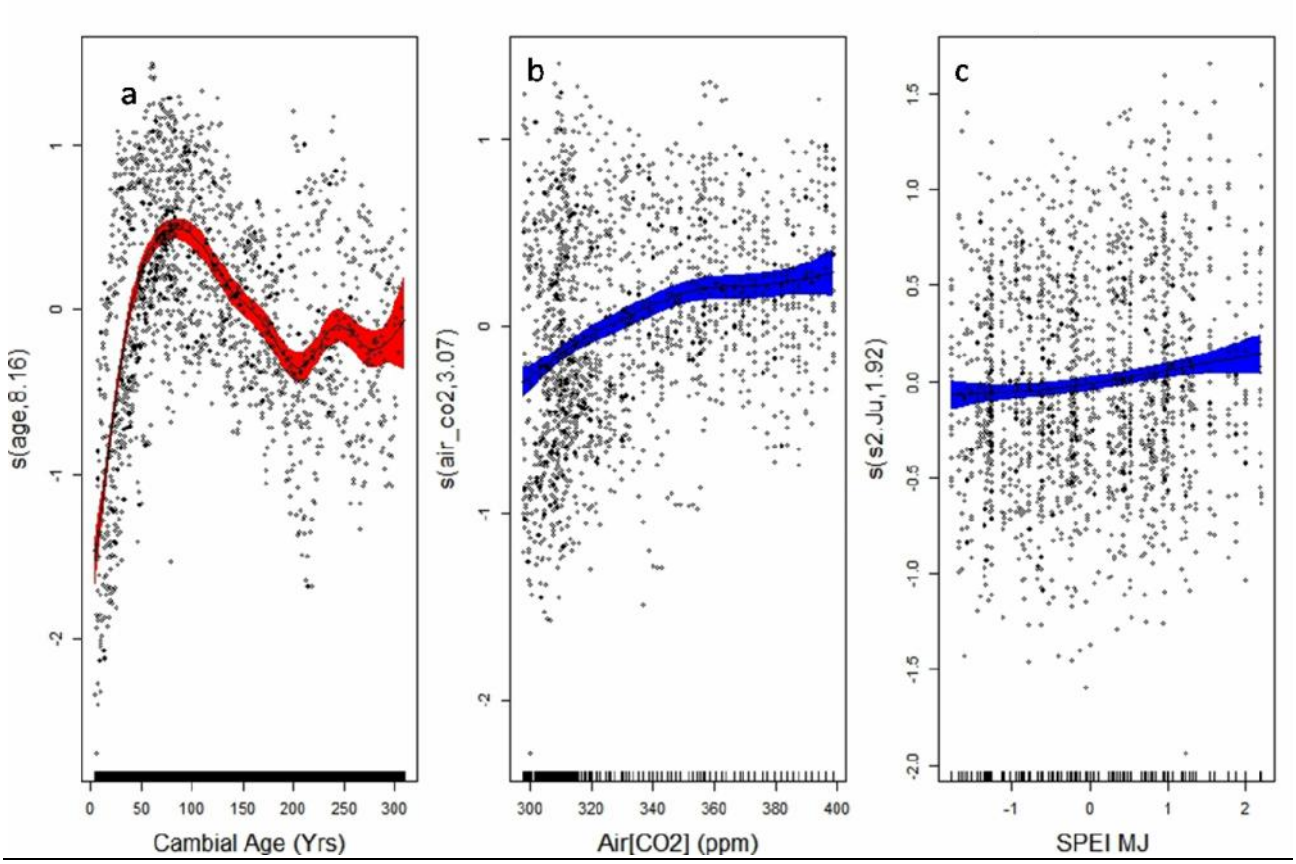


Fig. 7. GAM analysis of increment response to individual drivers. Generalized additive models (GAMs) results show the relationship between basal area increments (BAI) and environmental and biological factors remaining after the backward selection procedure: cambial age, atmospheric [CO2] and Standardizd Precipitation Evapotranspiration Index computed over May and June (SPEI MJ). Values on the *y*-axis indicate the independent effect of each covariate on basal area increments, as predicted by the model (continuous line) dimensionless and centered around 0, plus the estimated degree of freedom (edf). Points represent partial residuals from the fitted function and the shaded areas indicate the 95% prediction interval. The GAM model was applied to log-transformed BAI data, so as to correct for heteroscedasticity.

The effects of N deposition as a covariate were found not to be significant for either of the nitrogen forms (nor for their sum), maybe because of the low levels of pollutants which affect this site (Fig. supplementary material). Another study (Fenn et al. 2015), covering the entire California territory and considering 1706 permanent plots (33091 trees) found positive effects of N deposition on conifer diameter growth, but only above a threshold value of 15 kg/ha/yr, probably because of the damaging effects of ozone at lower N deposition rate.

4. Conclusions

From a methodological point of view, natural old-growth stands appear to have two major advantages when modelling tree growth response to environmental factors. First, they are less affected by inter-tree competition due to complex structure development or, at least, by the absence of synchronous consequences of thinning, and, second, they provide a very wide range in tree ages, so reducing the 'segment length curse' (Cook *et al.* 1995). The longer time span so covered could retain information referring to a pre-industrial world, essential when long-term environmental effects on trees growth, as well as age-related effects, are to be assessed, without the problems associated with confounding factors. At the same time, data from uneven-aged old-growth forests could be affected by a number of problems, first of all the possible link between growth rates and longevity, which would deserve further attention.

Also from a methodological point of view, the study demonstrated the need to combine dendroecological studies with a chronosquence approach, so as to be able to disentangle the global change effect from the co-occurring effects of tree ageing; despite its potential limitations (mainly due to the assumption of effect additivity, with no interaction between covariates) the GAM approach appears to be the best suited for such an analysis, provided that the number of covariates is kept to a minimum, with low cross-correlation.

The chronosequence approach coupled with the application of GAM models allowed us to highlight a strong increase in BAI at a constant age, which could be largely attributed to the effects of rising [CO2]. The spring/summer water availability, even if highly significant, looks likely to affect to a smaller extent the long-term trend in radial increments in this old-growth stand. These results show how the impact of global change, in absence of other limiting factors which normally could hide or negate its effect, has already modify tree growth rates since the pre-industrial era. This could mean that forest ecosystems have really the potential to exert a mitigation action on climate change, actively increasing over time their efficiency as carbon sinks (Popkin 2017), also in old-growth

1995 forests. On the other hand, it should be stressed that a similar pattern has not always been observed
1996 across the globe (Groenendijk *et al.* 2015); future studies should ascertain if such discrepancies can
1997 be explained by methodological biases or real differences between species and biomes.

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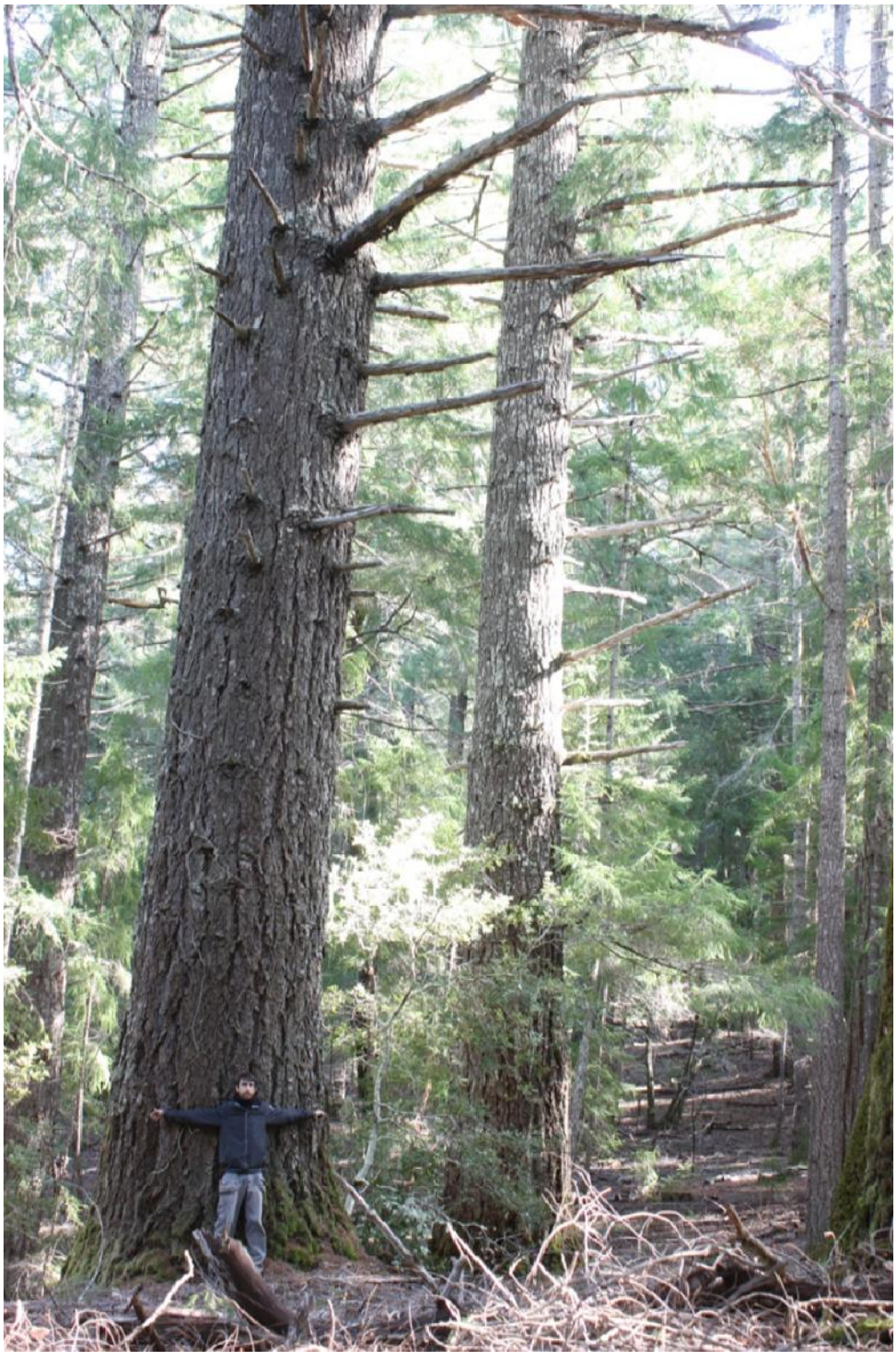

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5. Bibliography

ABER, JOHN D., CHRISTINE L. GOODALE, SCOTT V. OLLINGER, MARIE-LOUISE SMITH, ALISON H. MAGILL, MARY E. MARTIN, RICHARD A. HALLETT, and JOHN L. STODDARD. 2003. "Is Nitrogen Deposition Altering the Nitrogen Status of Northeastern Forests?" *BioScience* 53 (4): 375. doi:10.1641/0006-3568(2003)053[0375:INDATN]2.0.CO;2.

Aho, Ken, DeWayne Derryberry, and Teri Peterson. 2014. "Model Selection for Ecologists: The Worldview of AIC and BIC." *Ecology* 95 (March): 631–36. doi:10.1890/13-1452.1.

Ainsworth, Elizabeth A., and Stephen P. Long. 2005. "What Have We Learned from 15 Years of Free-Air CO2 Enrichment (FACE)? A Meta-Analytic Review of the Responses of Photosynthesis, Canopy Properties and Plant Production to Rising CO2." *New Phytologist* 165 (2): 351–71. doi:10.1111/j.1469-8137.2004.01224.x.

Babst, Flurin, M. Ross Alexander, Paul Szejner, Olivier Bouriaud, Stefan Klesse, John Roden, Philippe Ciais, et al. 2014. "A Tree-Ring Perspective on the Terrestrial Carbon Cycle." *Oecologia* 176 (2): 307–22. doi:10.1007/s00442-014-3031-6.

Beedlow, Peter A., E. Henry Lee, David T. Tingey, Ronald S. Waschmann, and Connie A. Burdick. 2013. "The Importance of Seasonal Temperature and Moisture Patterns on Growth of Douglas-Fir in Western Oregon, USA." *Agricultural and Forest Meteorology* 169. Elsevier B.V.: 174–85. doi:10.1016/j.agrformet.2012.10.010.

Biondi, Franco. 1999. "Comparing Tree-Ring Chronologies and Repeated timberiInventories as Forest Monitoring Tools." *Ecological Applications* 9 (1): 216–27. doi:10.1890/1051-0761(1999)009[0216:CTRCAR]2.0.CO;2.

Boettger, Tatjana, Marika Haupt, Kay Knöller, Stephan M. Weise, John S. Waterhouse, Katja T. Rinne, Neil J. Loader, et al. 2007. "Wood Cellulose Preparation Methods and Mass Spectrometric Analyses of ??13C, ??18O, and Nonexchangeable ??2H Values in Cellulose, Sugar, and Starch: An Interlaboratory Comparison." *Analytical Chemistry* 79 (12): 4603–12. doi:10.1021/ac0700023.

Boisvenue, Céline, and Steven W. Running. 2006. "Impacts of Climate Change on Natural Forest Productivity - Evidence since the Middle of the 20th Century." *Global Change Biology* 12 (5): 862–82. doi:10.1111/j.1365-2486.2006.01134.x.

Brienen, R J W, E Gloor, S Clerici, R Newton, L Arppe, A Boom, S Bottrell, et al. n.d. "Isotopes." *Nature Communications*. Springer US, 1–10. doi:10.1038/s41467-017-00225-z.

Brunel, J.P., G.R. Walker, C.D. Walker, J.C. Dighton, and A. Kennett-Smith. 1991. "Using Stable Isotopes of Water to Trace Plant Water Uptake." *International Symposium on the Use of Stable Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies*, 543–551. doi:10.2144/000114133.

Bunn, Andrew G. 2008. "A Dendrochronology Program Library in R (dplR)." *Dendrochronologia* 26 (2): 115–24. doi:10.1016/j.dendro.2008.01.002.

Bunn AG (2010). "Statistical and visual crossdating in R using the dplR library." *Dendrochronologia*, 28(4), pp. 251–258. ISSN 1125-7865, doi: 10.1016/j.dendro.2009.12.001

Camarero, J. Julio, Antonio Gazol, Jacques C. Tardif, and France Conciatori. 2015. “Attributing Forest Responses to Global-Change Drivers: Limited Evidence of a CO2-Fertilization Effect in Iberian Pine Growth.” *Journal of Biogeography* 42 (11): 2220–33. doi:10.1111/jbi.12590.

Carrer, Marco, and C Urbinati. 2006. “Long-Term Change in the Sensitivity of Tree- ring Growth to Climate Forcing in *Larix Decidua*.” *New Phytologist* 170 (iv): 861–72.

Cook, Edward R., Keith R. Briffa, David M Meko, Donald A Graybill, and Gary Funkhouser. 1995. “The ’segment Length Curse’ in Long Tree-Ring Chronology Development for Palaeoclimatic Studies.” *The Holocene* 5 (2): 229–37. doi:10.1177/095968369500500211.

Dawson, T E. 1998. “ Fog in the Californian Redwood Forest: Ecosystem Inputs and Use by Plants.” *Oecologia* 117: 476–85.

Dawson, Todd E., Stefania Mambelli, Agneta H. Plamboeck, Pamela H. Templer, and Kevin P. Tu. 2002. “Stable Isotopes in Plant Ecology.” *Annual Review of Ecology and Systematics* 33 (1): 507–59. doi:10.1146/annurev.ecolsys.33.020602.095451.

Dawson, Todd E., and John S Pate. 1996. “Seasonal Water Uptake and Movement in Root Systems of Australian Phraeatophytic Plants of Dimorphic Root Morphology: A Stable Isotope Investigation.” *Oecologia* 107 (1): 13–20. doi:10.1007/BF00582230.

Di Biase, Giampaolo, Gloria Falsone, Anna Graziani, Gilmo Vianello, and Livia Vittori Antisari. 2015. “Carbon Sequestration in Soils Affected By Douglas Fir Reforestation in Apennines (Northern Italy).” *Eqa-International Journal of Environmental Quality* 17: 1–11. doi:10.6092/issn.2281-4485/5208.

Dongmann, G, and H W Nürnberg. 1974. “On the Enrichment of H2180 in the Leaves of Transpiring Plants I T L Q E” 52: 1–2.

Esper, Jan, Edward R Cook, and Fritz H Schweingruber. 2002. “Low-Frequency Signals in Long Tree-Ring Chronologies for Reconstructing Past Temperature Variability.” *Science (New York, N.Y.)* 295 (5563). American Association for the Advancement of Science: 2250–53. doi:10.1126/science.1066208.

Esper, Jan, David C. Frank, Giovanna Battipaglia, Ulf Büntgen, Christopher Holert, Kerstin Treydte, Rolf Siegwolf, and Matthias Saurer. 2010a. “Low-Frequency Noise in δ13C and δ18O Tree Ring Data: A Case Study of Pinus Uncinata in the Spanish Pyrenees.” *Global Biogeochemical Cycles* 24 (4): 1–11. doi:10.1029/2010GB003772.

Esper, Jan, David C Frank, Giovanna Battipaglia, Ulf Büntgen, Christopher Holert, Kerstin Treydte, Rolf Siegwolf, and Matthias Saurer. 2010b. “Low - Frequency Noise in D 13 C and D 18 O Tree Ring Data : A Case Study of Pinus Uncinata in the Spanish Pyrenees” 24 (2): 1–11. doi:10.1029/2010GB003772.

Farquhar, G. D., L. A. Cernusak, and B. Barnes. 2006. “Heavy Water Fractionation during Transpiration.” *Plant Physiology* 143 (1): 11–18. doi:10.1104/pp.106.093278.

Farquhar, G D, J R Ehleringer, and K T Hubick. 1989. “Carbon Isotope Discrimination and Photosynthesis.” *Annual Review of Plant Physiology and Plant Molecular Biology*. doi:10.1146/annurev.pp.40.060189.002443.

Federal, Swiss, Universitat De Barcelona, J. Julio Camarero, Antonio Gazol, Juan Diego Galván, Gabriel Sangüesa-Barreda, and Emilia Gutiérrez. 2015. “Disparate Effects of Global-Change

Drivers on Mountain Conifer Forests: Warming-Induced Growth Enhancement in Young Trees vs. CO 2 Fertilization in Old Trees from Wet Sites." *Global Change Biology* 21 (2): 738–49. doi:10.1111/gcb.12787.

Fenn, Mark E, Jeremy S Fried, Haiganoush K Preisler, Andrzej Bytnerowicz, Susan Schilling, Sarah Jovan, and Olaf Kuegler. 2015. "REMEASURED FIA PLOTS REVEAL TREE-LEVEL DIAMETER GROWTH AND TREE MORTALITY IMPACTS OF NITROGEN DEPOSITION ON CALIFORNIA ' S FORESTS" 2013 (Time 2): 2013–16.

Francey, R. J., C. E. Allison, D. M. Etheridge, C. M. Trudinger, I. G. Enting, M. Leuenberger, R. L. Langenfelds, E. Michel, and L. P. Steele. 1999. "A 1000-Year High Precision Record of δ13C in Atmospheric CO2." *Tellus, Series B: Chemical and Physical Meteorology* 51 (2): 170–93. doi:10.1034/j.1600-0889.1999.t01-1-00005.x.

Frank, D. C., B. Poulter, M. Saurer, J. Esper, C. Huntingford, G. Helle, K. Treydte, et al. 2015. "Water-Use Efficiency and Transpiration across European Forests during the Anthropocene." *Nature Climate Change*, no. May. doi:10.1038/nclimate2614.

Giustini, Francesca, Mauro Brilli, and Antonio Patera. 2016. "Mapping Oxygen Stable Isotopes of Precipitation in Italy." *Journal of Hydrology: Regional Studies* 8. Elsevier B.V.: 162–81. doi:10.1016/j.ejrh.2016.04.001.

Gómez-Guerrero, Armando, Lucas C.R. R Silva, Miguel Barrera-Reyes, Barbara Kishchuk, Alejandro Velázquez-Martínez, Tomás Martínez-Trinidad, Francisca Ofelia Plascencia-Escalante, and William R. Horwath. 2013. "Growth Decline and Divergent Tree Ring Isotopic Composition (δ13C and δ18O) Contradict Predictions of CO2 Stimulation in High Altitudinal Forests." *Global Change Biology* 19 (6): 1748–58. doi:10.1111/gcb.12170.

Griffin, Daniel, and Kevin J Anchukaitis. 2014. "How Unusual Is the 2012 – 2014 California Drought ?," 9017–23. doi:10.1002/2014GL062433.1.

Guerrieri, R, Maurizio Mencuccini, L J Sheppard, M Saurer, M P Perks, P Levy, M a Sutton, Marco Borghetti, and J Grace. 2011. "The Legacy of Enhanced N and S Deposition as Revealed by the Combined Analysis of Delta 13C, Delta 18O and Delta 15N in Tree Rings." *Global Change Biology* 17 (5): 1946–62. doi:10.1111/j.1365-2486.2010.02362.x.

Harris, I., P. D. Jones, T. J. Osborn, and D. H. Lister. 2014. "Updated High-Resolution Grids of Monthly Climatic Observations - the CRU TS3.10 Dataset." *International Journal of Climatology* 34 (3): 623–42. doi:10.1002/joc.3711.

Hastie, T. J., and R. J. Tibshirani. 1990. "Generalized Additive Models." *Monographs on Statistics and Applied Probability*. doi:10.1016/j.csda.2010.05.004.

IPCC. 2014. "Climate Change 2014 Synthesis Report Summary Chapter for Policymakers." *Ipcc*, 31. doi:10.1017/CBO9781107415324.

Kaufmann, M.R. 1996. To live fast or not: growth, vigor and longevity of old-growth ponderosa pine and lodgepole pine trees. Tree Physiology 16:139-144.

Keenan, Trevor F, David Y Hollinger, Gil Bohrer, Danilo Dragoni, J William Munger, Hans Peter Schmid, and Andrew D Richardson. 2013. "Increase in Forest Water-Use Efficiency as Atmospheric Carbon Dioxide Concentrations Rise." *Nature* 499 (7458): 324–27. doi:10.1038/nature12291.

Korner, C. 2005. “Carbon Flux and Growth in Mature Deciduous Forest Trees Exposed to Elevated CO2.” *Science* 309 (5739): 1360–62. doi:10.1126/science.1113977.

Leavitt, Steven W. 2010. “Tree-Ring C-H-O Isotope Variability and Sampling.” *The Science of the Total Environment* 408 (22): 5244–53. doi:10.1016/j.scitotenv.2010.07.057.

Lee, E. Henry, Peter A. Beedlow, Ronald S. Waschmann, David T. Tingey, Charlotte Wickham, Steve Cline, Michael Bollman, and Cailie Carlile. 2016. “Douglas-Fir Displays a Range of Growth Responses to Temperature, Water, and Swiss Needle Cast in Western Oregon, USA.” *Agricultural and Forest Meteorology* 221. Elsevier B.V.: 176–88. doi:10.1016/j.agrformet.2016.02.009.

Leonardi, Stefano, Tiziana Gentilesca, Rossella Guerrieri, Francesco Ripullone, Federico Magnani, Maurizio Mencuccini, Twan V. Noije, and Marco Borghetti. 2012. “Assessing the Effects of Nitrogen Deposition and Climate on Carbon Isotope Discrimination and Intrinsic Water-Use Efficiency of Angiosperm and Conifer Trees under Rising CO 2 Conditions.” *Global Change Biology* 18 (9): 2925–44. doi:10.1111/j.1365-2486.2012.02757.x.

Lévesque, Mathieu, Rolf Siegwolf, Matthias Saurer, Britta Eilmann, and Andreas Rigling. 2014. “Increased Water-Use Efficiency Does Not Lead to Enhanced Tree Growth under Xeric and Mesic Conditions.” *New Phytologist* 203 (1): 94–109. doi:10.1111/nph.12772.

Linares, Juan Carlos, Jesús Julio Camarero, and José Antonio Carreira. 2010. “Competition Modulates the Adaptation Capacity of Forests to Climatic Stress: Insights from Recent Growth Decline and Death in Relict Stands of the Mediterranean Fir Abies Pinsapo.” *Journal of Ecology* 98 (3): 592–603. doi:10.1111/j.1365-2745.2010.01645.x.

Magnani, Federico, Maurizio Mencuccini, Marco Borghetti, Paul Berbigier, Frank Berninger, Sylvain Delzon, Achim Grelle, et al. 2007. “The Human Footprint in the Carbon Cycle of Temperate and Boreal Forests.” *Nature* 447 (7146): 848–50. doi:10.1038/nature05847.

Marshall, D D, and R O Curtis. 2002. “Levels-of-Growing-Stock Cooperative Study in Douglas-Fir: Report No. 15 - Hoskins: 1963-1998.” *Research Paper Pacific Northwest Research Station, USDA Forest Service*, no. PNW-RP-537: 80.

McCarroll, Danny, and Neil J. Loader. 2004. “Stable Isotopes in Tree Rings.” *Quaternary Science Reviews* 23 (7–8): 771–801. doi:10.1016/j.quascirev.2003.06.017.

McMahon, Sean M, Geoffrey G Parker, and Dawn R Miller. 2010. “Evidence for a Recent Increase in Forest Growth.” *Proceedings of the National Academy of Sciences of the United States of America* 107 (8): 3611–15. doi:10.1073/pnas.0912376107.

Medlyn, B. E., F. -W. Badeck, D. G. G. De Pury, C. V. M. Barton, M. Broadmeadow, R. Ceulemans, P. De Angelis, et al. 1999. “Effects of Elevated [CO2] on Photosynthesis in European Forest Species: A Meta-Analysis of Model Parameters.” *Plant, Cell & Environment* 22 (12): 1475–1495. doi:10.1046/j.1365-3040.1999.00523.x.

Nair, Richard K F, Micheal P Perks, Andrew Weatherall, Elizabeth M Baggs, and Maurizio Mencuccini. 2015. “Does Canopy Nitrogen Uptake Enhance Carbon Sequestration by Trees?” *Global Change Biology*, September. doi:10.1111/gcb.13096.

Nehrbass-Ahles, Christoph, Flurin Babst, Stefan Klesse, Magdalena Nötzli, Olivier Bouriaud, Raphael Neukom, Matthias Dobbertin, and David Frank. 2014. “The Influence of Sampling Design on Tree-Ring-Based Quantification of Forest Growth.” *Global Change Biology* 20 (9).

doi:10.1111/gcb.12599.

Norby, Richard J, Jeffrey M Warren, Colleen M Iversen, Belinda E Medlyn, and Ross E McMurtrie. 2010. “CO2 Enhancement of Forest Productivity Constrained by Limited Nitrogen Availability.” *Proceedings of the National Academy of Sciences of the United States of America* 107 (45): 19368–73. doi:10.1073/pnas.1006463107.

Peñuelas, Josep, Josep G. Canadell, and Romà Ogaya. 2011. “Increased Water-Use Efficiency during the 20th Century Did Not Translate into Enhanced Tree Growth.” *Global Ecology and Biogeography* 20 (4): 597–608. doi:10.1111/j.1466-8238.2010.00608.x.

Perakis, Steven S., and Emily R. Sinkhorn. 2011. “Biogeochemistry of a Temperate Forest Nitrogen Gradient.” *Ecology* 92 (7): 1481–91. doi:10.1890/10-1642.1.

Peters, Richard L., Peter Groenendijk, Mart Vlam, and Pieter a. Zuidema. 2015. “Detecting Long-Term Growth Trends Using Tree Rings: A Critical Evaluation of Methods.” *Global Change Biology*, n/a-n/a. doi:10.1111/gcb.12826.

Phillips, Nathan G., Thomas N. Buckley, and David T. Tissue. 2008. “Capacity of Old Trees to Respond to Environmental Change.” *Journal of Integrative Plant Biology* 50 (11): 1355–64. doi:10.1111/j.1744-7909.2008.00746.x.

Piovesan, Gianluca, Franco Biondi, Aalfredo Di Filippo, Alfredo Alessandrini, and Maurizio Maugeri. 2008. “Drought-Driven Growth Reduction in Old Beech (Fagus Sylvatica L.) Forests of the Central Apennines, Italy.” *Global Change Biology* 14 (6): 1265–81. doi:10.1111/j.1365-2486.2008.01570.x.

Poage, Nathan J, and John C Tappeiner, II. 2002. “Long-Term Patterns of Diameter and Basal Area Growth of Old-Growth Douglas-Fir Trees in Western Oregon.” *Canadian Journal of Forest Research* 32: 1232–43. doi:10.1139/x02-045.

Rehfeldt, Gerald E., Barry C. Jaquish, Javier Lòpez-Upton, Cuauhtémocmoc Sáenz-Romero, J. Bradley St Clair, Laura P. Leites, and Dennis G. Joyce. 2014. “Comparative Genetic Responses to Climate for the Varieties of Pinus Ponderosa and Pseudotsuga Menziesii: Realized Climate Niches.” *Forest Ecology and Management* 324. Elsevier B.V.: 126–37. doi:10.1016/j.foreco.2014.02.035.

Ripullone, Francesco, Marco Lauteri, Giacomo Grassi, Mariana Amato, and Marco Borghetti. 2004. “Variation in Nitrogen Supply Changes Water-Use Efficiency of Pseudotsuga Menziesii and Populus X Euroamericana; a Comparison of Three Approaches to Determine Water-Use Efficiency.” *Tree Physiology* 24 (6): 671–79.

Rita, Angelo, Marco Borghetti, Luigi Todaro, and Antonio Saracino. 2016. “Interpreting the Climatic Effects on Xylem Functional Traits in Two Mediterranean Oak Species: The Role of Extreme Climatic Events.” *Frontiers in Plant Science* 7 (August): 1–11. doi:10.3389/fpls.2016.01126.

Roden, John, and Rolf Siegwolf. 2012. “Is the Dual-Isotope Conceptual Model Fully Operational?” *Tree Physiology* 32 (10): 1179–82. doi:10.1093/treephys/tps099.

Rozanski, Kazimierz, Luis Araguas-araguas, and Roberto Gonfiantini. 2016. “Relation Between Long-Term Trends of Oxygen-18 Isotope Composition of Precipitation and Climate Author (S): Kazimierz Rozanski , Luis Araguás-Araguás and Roberto Gonfiantini Published by : American Association for the Advancement of Science Stable URL :” 258 (5084): 981–85.

Ryan, Mg, D Binkley, and Jh Fownes. 1997. “Age-Related Decline in Forest Productivity: Pattern and Process.” *Advances in Ecological Research*.

Ryan, Michael G., Nathan Phillips, and Barbara J. Bond. 2006. “The Hydraulic Limitation Hypothesis Revisited.” *Plant, Cell and Environment* 29 (3): 367–81. doi:10.1111/j.1365-3040.2005.01478.x.

Ryan, Michael G., and Barbara J. Yoder. 1997. “Hydraulic Limits to Tree Height and Tree Growth.” *BioScience* 47 (4): 235–42. doi:10.2307/1313077.

Saurer, Matthias, Rolf T. W. Siegwolf, and Fritz H. Schweingruber. 2004. “Carbon Isotope Discrimination Indicates Improving Water-Use Efficiency of Trees in Northern Eurasia over the Last 100 Years.” *Global Change Biology* 10 (12): 2109–20. doi:10.1111/j.1365-2486.2004.00869.x.

Scheidegger, Y., M. Saurer, M. Bahn, and R. Siegwolf. 2000. “Linking Stable Oxygen and Carbon Isotopes with Stomatal Conductance and Photosynthetic Capacity: A Conceptual Model.” *Oecologia* 125 (3): 350–57. doi:10.1007/s004420000466.

Sitch, Stephan, C. Huntingford, N. Gedney, P. E. Levy, M. Lomas, S. L. Piao, R. Betts, et al. 2008. “Evaluation of the Terrestrial Carbon Cycle, Future Plant Geography and Climate-Carbon Cycle Feedbacks Using Five Dynamic Global Vegetation Models (DGVMs).” *Global Change Biology* 14 (9): 2015–39. doi:10.1111/j.1365-2486.2008.01626.x.

Sternberg, Leonel Da Silveira Lobo O.Reilly. 2009. “Oxygen Stable Isotope Ratios of Tree-Ring Cellulose: The next Phase of Understanding.” *New Phytologist* 181 (3): 553–62. doi:10.1111/j.1469-8137.2008.02661.x.

Treydte, Kerstin, Sonja Boda, Elisabeth Graf Pannatier, Patrick Fonti, David Frank, Bastian Ullrich, Matthias Saurer, et al. 2014. “Seasonal Transfer of Oxygen Isotopes from Precipitation and Soil to the Tree Ring : Source Water versus Needle Water Enrichment.”

Vicente-Serrano, Sergio M., Santiago Beguería, and Juan I. López-Moreno. 2010. “A Multiscalar Drought Index Sensitive to Global Warming: The Standardized Precipitation Evapotranspiration Index.” *Journal of Climate* 23 (7): 1696–1718. doi:10.1175/2009JCLI2909.1.

Vicente-Serrano, Sergio M., J. Julio Camarero, and Cesar Azorin-Molina. 2014. “Diverse Responses of Forest Growth to Drought Time-Scales in the Northern Hemisphere.” *Global Ecology and Biogeography* 23 (9): 1019–30. doi:10.1111/geb.12183.

Vitas, A., and K. Žeimavičius. 2006. “Trends of Decline of Douglas Fir in Lithuania : Dendroclimatological Approach.” *Baltic Forestry* 12 (2): 200–208.

Walker, Lawrence R., David a. Wardle, Richard D. Bardgett, and Bruce D. Clarkson. 2010. “The Use of Chronosequences in Studies of Ecological Succession and Soil Development.” *Journal of Ecology* 98 (4): 725–36. doi:10.1111/j.1365-2745.2010.01664.x.

Warren, Cr, Jf McGrath, and Ma Adams. 2001. “Water Availability and Carbon Isotope Discrimination in Conifers.” *Oecologia* 127 (4): 476–86. doi:10.1007/s004420000609.

Williams, David G., R. David Evans, Jason B. West, and James R. Ehleringer. 2007. *Stable Isotopes as Indicators of Ecological Change*. *Terrestrial Ecology*. Vol. 1. doi:10.1016/S1936-7961(07)01024-X.

Wood, Simon N. 2014. “Package ’ Mgcv ’.” doi:10.1186/1471-2105-11-11.Bioconductor.

Wood, Simon N. 2017. “Package ‘ Mgcv .’”

Wood, Simon N. 2006. *Generalized Additive Models: An Introduction with R*. Chapman and Hall/CRC.

Wood, Simon N. 2008. “Fast Stable Direct Fitting and Smoothness Selection for Generalized Additive Models.” *Journal of the Royal Statistical Society. Series B: Statistical Methodology* 70 (3): 495–518. doi:10.1111/j.1467-9868.2007.00646.x.

Zhao, Maosheng, and Steven W Running. 2010. “Drought-Induced Reduction in Global Terrestrial Net Primary Production from 2000 Through 2009.” *Science* 329 (5994): 940–43. doi:10.1126/science.1192666.

Zweifel, Roman, Lukas Zimmermann, Fabienne Zeugin, and David M. Newbery. 2006. “Intra-Annual Radial Growth and Water Relations of Trees: Implications towards a Growth Mechanism.” *Journal of Experimental Botany* 57 (6): 1445–59. doi:10.1093/jxb/erj125.

Conclusions

Given the widely observed impact of the so-called global change on forest productivity, and the associated eco-physiological trend, a proper understanding of the dynamic response to the individual main drivers involved appears to be crucial, both to project the future role of forest as a sink or source of carbon, and to try and adapt forest management choices to a changing world.

In this perspective, using tree ring width and isotopic signature analysis, we tried to disentangle which forcing factors have determined the long-term trend in growth observed in two Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) age sequences: an even-aged chronosequence in Vallombrosa, Italy and a natural old-growth age sequence in Angelo Coast Range Reserve, California.

In chapter one we focused on last century's growth variations in Vallombrosa, analyzed after decoupling it from age-size related effects. The resulting trend displays a multi-decadal oscillation and a recent decrement of basal area increments (BAI) of the order of 22.9%. Through the application of generalized additive models (GAMs) we were able to assess, among a number of

potentially relevant environmental factors, which ones determined this trend. The model applied describes 37% of log(BAI) variability and the combination of covariates that were found to be significant includes N deposition (in the oxidized form) and summer water availability for both the growing season of ring formation and the previous year, all positively related with growth at a P level <0.001. On the other hand atmospheric CO_2 concentration, despite the significant effect on BAI variations, displays a non-monotonic shape of the relationship which could be most likely determined by a possible problem with the model (either concurvity or failure to consider all important independent variables), rather than by a meaningful biological process alone, such as a progressive down- regulation in photosynthetic response, or a shift in resources allocation. However it is possible to state that the BAI declining trend in recent times should be attributed, at least partially, to an effect of decreasing N deposition and an increase in summer water deficit.

In the second chapter, considering the same chronosequence, isotopic analyses were employed to investigate the underlying variations in tree physiological response. More specifically, the $\delta^{13}C$ and $\delta^{18}O$ were analyzed, also in a dual isotope perspective, so as to clarify which drivers have affected the observed trend in intrinsic water use efficiency (iWUE), whether an increase in photosynthetic rates or a reduction in stomatal conductance, or both. Once decupled from age-related effects, the pattern over time in water use efficiency shows an increase of the order of 17.3 % in the period included between 1960-1980, followed by a less pronounced increase in the following years; at the same time the age effect *per se* displayed a pronounced rise, suggesting a possible progressive increase in stomatal control, in agreement with the hydraulic limitation hypothesis (HLH). The GAMs analysis of iWUE dynamics was found to explain 80% of its overall variability; for what concerns the single covariates effects, CO_2 was found to be positively related with iWUE, as well as NOy deposition, as already generally observed in other studies; In addition also the water availability of the previous summer has a fully significant effect. Considering $\delta^{18}O$ dynamics, on the contrary, only 46% of overall signal variability was explained by environmental variables. An inverse relationship with precipitation and a positive one with annul maximum temperature

(possibly because of its link with VPD) were detected, confirming to a certain extent the goodness of the signal recorded in tree-rings, although the possibility cannot be ruled out that a decreasing trend in the isotopic signature of the source water composition could have conditioned the analysis results. No effects of age variation were detected. The simultaneous consideration of both isotopes together in the dual-isotope approach, finally, allowed us to demonstrate that A_{max} and not g_s appears to have driven the trend in iWUE over the second part of the last century. This seems to agree with the hypothesis that the observed pattern in iWUE could have been determined by an initial stimulation of assimilation driven by N deposition in combination with the CO_2 increase, successively reversed over the last decades by the reduction in N deposition, coupled with a possible influence of drought and competition.

The last chapter should be considered as an explorative study, as the chronosequence is constituted by only a few trees (n=18); having said that, the analysis presented has the main advantage of comprising a wide range of ages, spanning all the way back to pre-industrial times, and to be located in a natural old growth forest, less prone to inter-tree competition. The results show a strong increase in BAI since the pre-industrial period, with a 59.5% increase for the last 60 years alone, especially as a result of the atmospheric CO_2 concentration effect. Moreover, the shape of age-related growth trends observed in the different age classes when taking a diachronic perspective, suggest a gradual anticipation in the culmination of increments, as would be expected as a result of increasing site fertility over time. This look like an additional confirmation of the substantial global change impact on Douglas fir growth in this old-growth stand.

At last, summarizing the main finding of this study:

1. The age-related pattern in iWUE suggests that the observed ontogenetic dynamics in BAI could be the result of an increase in g_s control as trees grow taller; this is likely the case in the Vallombrosa chronosequnce, where the increment in tree height is not yet stabilized even in oldest age class (data not shown), and possibly also in the Angelo forest. On the other hand, this runs

against the absence of such an age effect in the $\delta^{18}O$ signal, even if the known relevance of source water isotopic signature and the general lack of detailed information its variability lessens the potential suitability of this proxy in a long term view. Nevertheless, the effect of age on isotopic C composition should be interpreted with great care when using iWUE to infer global change effects, as there could be the risk to overestimating the relevance of stomatal regulation.

2. The analysis at both sites demonstrates the importance of water availability in the summer period as a limiting factor for the growth of this species in Mediterranean environments, affecting both increments and eco-physiological traits. The increase in atmospheric CO_2, which shows a positive contribution to the BAI trend at the Angelo site, does not display the same relationship in Vallombrosa. On the other hand, this covariate was found to strongly affect iWUE at the Italian site, by increasing leaf photosynthetic capacity as demonstrated by the dual isotope approach. Also the effect of atmospheric nitrogen deposition shows marked differences among sites. While at Angelo site no effect was observed (possibly as a result of the substantially lower level in N deposition), in Vallombrosa this covariate shows the same positive effect on both dependent variables (BAI and iWUE), probably because both are sensitive to A_{max} and leaf N contents. This consistency between the two analyses increases the reliability of our conclusions that N deposition - at this site at least - should be viewed as an influential factor affecting stem growth through its effects on photosynthetic capacity, highlighting the importance of considering N deposition when global change effects on forest growth and function are to be assessed.